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(54) Title: ANALYSIS METHOD

(57) Abstract: This invention relates to novel methods for the identification of genes and gene products that are implicated in certain disease states. According to the invention, there is provided a method for the identification of a gene that is implicated in a specific disease or physiological condition, said method comprising the steps of comparing: i) the transcriptome or proteome of a first specialised cell type that is implicated in the disease or condition under first and second experimental conditions; with ii) the transcriptome or proteome of a second specialised cell type under said first and said second experimental conditions; and identifying as a gene implicated in the disease or physiological condition, a gene that is differentially regulated in the two specialised cell types under the first and second experimental conditions. The invention also relates to novel genes and gene products identified using these methods.

Analysis method

This invention relates to novel methods for the identification of genes and gene products that are implicated in certain disease states. The invention also relates to novel genes and gene products identified using these methods.

All publications, patents and patent applications cited herein are incorporated in full by reference.

One of the central goals in the field of gene expression is to understand and elucidate the relationship between a particular disease state and the gene expression pattern that defines and/or causes this disease state. Research has concentrated on differences in expression patterns between diseased and healthy tissues to elucidate the physiological mechanisms of disease. Identified differences in expression patterns provide putative points for therapeutic intervention to reverse the disease phenotype. These differences also provide markers that are useful for diagnosis, and identify proteins for further investigation as agents implicated in the disease in question.

Conventional methods for the elucidation of mechanisms of disease tend to concentrate on the correlation of a disease state with altered levels of a particular protein. Such methods include techniques of immunohistochemistry, the study of differential mRNA expression and the sequence analysis of particular proteins to identify mutations that are associated with a certain disease state.

Recently, research has concentrated on analysis of the transcriptomes of organisms and cell types that are considered to be of scientific interest. By "transcriptome" is meant the exact set of transcripts that are expressed in a cell. The emerging field of nucleic acid arrays is one field in which a large number of powerful tools are being generated for the study of transcriptome variation between different tissue types. These tools are based on techniques originally pioneered by Schena *et al.*, 1995 (Science 270: 467-470) and Fodor *et al.*, 1991 (Science 251, 767-773) and facilitate the evaluation of variations in DNA or RNA sequences and of variations in expression levels from tissue samples and allow the identification and genotyping of mutations and polymorphisms in these sequences. The power of one such technique has recently been demonstrated by Perou *et al.*, (Nature, 2000, 406:747-752), who generated molecular portraits of the transcriptomes of human breast tumours.

Over recent years, the so-called "genomics revolution" has allowed access to large portions of whole genomes, including the human genome. The amount of sequence information now available considerably facilitates the analysis of the results of experiments that aim to elucidate the differences between gene expression in diseased and healthy tissues. As this information increases in scope and becomes more readily available, the study of the molecular mechanism of disease, and the elucidation of techniques for combatting these diseases will be considerably facilitated.

However, there are notable disadvantages associated with all methods that are currently employed for the

analysis of human disease. Many methods currently employed utilise established cell lines. Because these cells have been manipulated to allow their immortalisation in cell culture, the physiological situation in these cells is not considered by the present inventors to be generally representative of the authentic situation in equivalent cells *in vivo*. Furthermore, most of these methods tend to utilise a global strategy for intervention, often ignoring the intricacies in gene expression that exist between different tissues. There thus remains a great need for the establishment of novel methods for the analysis of gene expression.

According to the invention, there is provided a method for the identification of a gene that is implicated in a specific disease or physiological condition, said method comprising the steps of:

a) comparing:

i) the transcriptome or proteome of a first specialised cell type that is implicated in the disease or condition under first and second experimental conditions; with

ii) the transcriptome or proteome of a second specialised cell type under said first and said second experimental conditions; and

b) identifying as a gene implicated in the disease or physiological condition, a gene that is differentially regulated in the two specialised cell types under the first and second experimental conditions.

Using this method, genes have been identified that respond to perturbations of cell physiology in a cell-specific rather than a generic fashion. The method of the invention exhibits significant advantages over conventional methods of identifying genes that are implicated in disease.

Various groups have previously investigated mechanisms of physiological regulation, by comparing gene expression levels in the presence and absence of a physiological stimulus or challenge. Genes identified in a particular cell type as being expressed at different levels under different conditions are implicated as components of a pathway that is responsive to the altered conditions, or that is regulated differently under the altered conditions. However, these methods exhibit a tendency to ignore patterns of gene expression that are physiologically relevant. This inclination is considered to result from a prejudice in the art that dictates that cells respond to changes in certain physiological conditions in a generic fashion, rather than in a cell specific fashion.

By "implicated in a specific disease or physiological condition" is meant that the gene has been found to possess a distinct role in a pathway that is involved in susceptibility to, generation of or maintenance of a particular disease phenotype or physiological condition. As will be apparent to the skilled reader, any

point in any pathway may be the unique point at which a cell departs from the normal physiological response and generates a disease phenotype. Often the effect that is manifested as a disease is the result of a mutation event, in which a mutation occurs in the sequence of a gene encoding a protein that functions in a relevant physiological pathway.

There are numerous examples of diseases and conditions that may be studied using the method of the invention. Such pathological conditions include those that result from a change in the intrinsic nature of a cell (usually genetic) or from a change in the cellular microenvironment, either of which might be recapitulated in a laboratory setting. The methods may be applied to any disease or condition that is manifested in, or is generated in a specific cell type.

Examples of such conditions include changes in the cellular microenvironment, exposure to hormones, growth factors, cytokines, chemokines, inflammatory agents, toxins, metabolites, pH, pharmaceutical agents, hypoxia, anoxia, ischemia, imbalance of any plasma-borne nutrient [including glucose, amino acids, co-factors, mineral salts, proteins and lipids], osmotic stress, temperature [hypo and hyper-thermia], mechanical stress, irradiation [ionising or non-ionising], cell-extracellular matrix interactions, cell-cell interactions, accumulations of foreign or pathological extracellular components, intracellular and extracellular pathogens [including bacteria, viruses, fungi and mycoplasma] and genetic perturbations [both epigenetic or mediated by mutation or polymorphism].

Examples of such diseases include cardiovascular disease, atherosclerosis, inflammatory conditions (including rheumatoid arthritis), cancer, ischemic disease, asthma, hematopoietic disorders, neurological diseases including Parkinson's and Alzheimer's diseases, infectious disease and allergies.

One particular physiological response that has been used herein to illustrate the invention is the cellular response to hypoxia. The term "hypoxia" is intended to refer to an environment of reduced oxygen tension, as compared to the normal physiological environment for a particular organism, which is termed "normoxia". The prejudice in this technical field presents the view that there is a general, ubiquitous response to hypoxia, mediated primarily at the level of mRNA (transcriptional initiation and post-transcriptional stabilisation).

In a variety of human diseases, cells are exposed to conditions of low oxygen tension, usually as a result of poor oxygen supply to the diseased area. For instance, tissue oxygenation plays a significant regulatory role in both apoptosis and in angiogenesis (Bouck *et al*, 1996, Adv. Cancer Res. 69:135-174; Bunn *et al*, 1996, Physiol. Rev. 76:839-885; Dor *et al*, 1997, Trends Cardiovasc. Med., 7:289-294; Carmeliet *et al*, 1998, Nature 394:485-490). Apoptosis (see Duke *et al*, 1996, Sci. American, 80-87 for review) and growth arrest occur when cell growth and viability are reduced due to oxygen deprivation. Angiogenesis

(i.e. blood vessel growth, vascularization), is stimulated when hypooxygenated cells secrete factors that stimulate proliferation and migration of endothelial cells in an attempt to restore oxygen homeostasis (for review see Hanahan *et al*, 1996, Cell, 86:353-364).

Ischaemic disease pathologies involve a decrease in the blood supply to a bodily organ, tissue or body part generally caused by constriction or obstruction of the blood vessels. For example, solid tumours typically have a disorganised blood supply, leading to hypoxic regions. Other disease conditions involving hypoxia include stroke, atherosclerosis, retinopathy, acute renal failure, myocardial infarction, stroke and hair loss. Therefore, apoptosis and angiogenesis as induced by the ischaemic condition are also considered to be involved in these disease states. It is generally considered that understanding the mechanism by which cells respond to these diseases may be the key to the disease pathology and thus relevant to disease treatment.

In a different but related approach, it is now recognised that angiogenesis is necessary for tumour growth and that retardation of this process provide a useful tool in controlling malignancy and retinopathies. For example, neoangiogenesis is seen in many forms of retinopathy and in tumour growth. The ability to be able to induce tumourigenic cells to undergo apoptosis is an extremely desirable goal; particularly in the cancer field, it has been observed that apoptosis and angiogenesis-related genes provide potent therapeutic targets. It has also been observed that hypoxia plays a critical role in the selection of mutations that contribute to more severe tumourigenic phenotypes (Graeber *et al.*, 1996 Nature, 379(6560):88-91).

Early in the history of this field it was discovered that a transcription factor, HIF-1alpha, is ubiquitously present in cells and is responsible for the induction of a number of genes in response to hypoxia. This protein is considered a master regulator of oxygen homeostasis (see, for example, Semenza, (1998) Curr. Op. Genetics and Dev. 8:588-594). Where HIF1alpha is genetically knocked out, the hypoxia-inducible transcription of virtually all glycolytic enzymes has been shown to be inhibited. Glycolysis is an essential process which goes on in all mammalian cells. This finding is therefore consistent with previous work showing that when cells are exposed to conditions of hypoxia, they up-regulate glycolytic enzymes to enable ATP production, since oxidative phosphorylation is no longer feasible under conditions of low oxygen (Webster (1987) *Mol.Cell.Biochem*, 77: 19-28). Further support for a critical and general role of HIF1alpha in the hypoxic response is demonstrated by the knockout mouse, which dies at day 10.5 of gestation. The same is true of the knockout of the ARNT protein, the dimerisation partner of HIF1alpha.

For the first time, it is demonstrated herein that different tissues and cell types exhibit a very different response to hypoxia, at the level of the induction and repression of gene expression. This has allowed the

detailed elucidation of the mechanism of this particular physiological response, so paving the way for the development of improved therapeutic agents that target components of the response pathway in particular tissues. Although conventional approaches to the analysis of this mechanism have successfully identified numerous genes, because of the universal prejudice in the art that these components will all be induced/repressed similarly in all cell types, all the approaches suggested have hitherto been limited to the design of therapeutic agents that act in a global fashion.

The methods of the present invention therefore extend and add to previous work performed in this field, in that the discoveries made now allow the design of agents that target the hypoxic response in specific tissues. For example, it is known that brain and heart tissues die very rapidly after ischaemic insult. By using the method of the invention, it is quite possible that these tissues will be found to share common features in their response to hypoxia, that is different from other cell types. This might allow, for example, the design of a combination cardioprotective and neuroprotective agent effective against this subset of body tissues. Alternatively, the hypoxic response in these tissues might be found to be quite different. This information would then be taken into account when designing therapeutic countermeasures, in that an agent would be designed for the unique neurological or cardiological tissue concerned.

The method of the invention involves the comparison of the transcriptomes or proteomes of at least two specialised cell types under two different physiological or genetic conditions. By "transcriptome" is meant the exact set of transcripts that are expressed in a cell. The transcriptome thus has a qualitative element (the identity of individual gene transcripts) and a quantitative element (the proportion of each unique transcript in the total number of individual transcripts present in the cell at a particular moment). By "proteome" is meant the exact set of protein molecules that are expressed in a cell.

By "specialised cell type" is meant a cell type that has a restricted biochemical capacity and that can be unambiguously identified as possessing a unique set of biochemical and physiological functions. Preferably, the specialised cells are primary cells, and not cell lines or whole body tissues. Primary cells are cells that cannot proliferate indefinitely in culture. Primary cells can be derived from adult tissue, or from embryo tissue that is differentiated in culture to an adult cell or to a precursor of an adult cell that displays specialised characteristics.

Examples of preferred specialised cell types include cardiomyocytes, endothelial cells, sensory neurons, motor neurons, CNS neurons (all types), astrocytes, glial cells, schwann cells, mast cells, eosinophils, smooth muscle cells, skeletal muscle cells, pericytes, lymphocytes, tumor cells, monocytes, macrophages, foamy macrophages, granulocytes, synovial cells / synovial fibroblasts, epithelial cells (varieties from all

tissues/ organs). Examples of other suitable specialised cell types include vascular endothelial cells, smooth muscle cells (aortic, bronchial, coronary artery, pulmonary artery, etc), skeletal muscle cells, cardiomyocyte cells, fibroblasts (many types, such as synovial), keratinocytes, hepatocytes, dendritic cells, astrocytes, neurone cells (including mesencephalic, hippocampal, striatal, thalamic, hypothalamic, olfactory bulb, substantia nigra, locus coeruleus, cortex, dorsal root ganglia, superior cervical ganglia, sensory, motor, cerebellar cells), neutrophils, eosinophils, basophils, mast cells, monocytes, macrophage cells, erythrocytes, megakaryocytes, hematopoietic progenitor cells, hematopoietic pluripotent stem cells, any stem cells, any progenitor cells, epithelial cells, melanocytes, osteoblasts, osteoclasts, stromal cells, purkinje cells, T-cells, B-cells, synovial cells, pancreatic islet cells (alpha and beta), leukemia cells, lymphoma cells, tumour cells, retinal cells, adrenal chromaffin cells. As will be apparent to the skilled reader, it is not here possible to provide an exhaustive list of specialised cell types that may be studied according to the methods of the present invention.

Intended as being included within the method of the invention is the possibility of using, as two different specialised cell types, two different physiological states of the same cell type, for example, activated and resting macrophages.

The transcriptomes of the specialised cell types are compared under different experimental conditions. The term "experimental conditions" is used broadly in this context and is intended to embrace any physiological or genetic conditions to which a cell type may be exposed. The intention of the method is to compare the transcriptomes or proteomes of the cell types under different experimental conditions that have a physiological relevance. Accordingly, the state of the transcriptome or proteome under one set of experimental conditions will generally act as a control against which the transcriptome or proteome may be compared under a second set of experimental conditions. Any distinct physiologically-relevant conditions may therefore be of interest.

Examples of suitable physiological experimental conditions include conditions under which the cell is submitted to a physiological, mechanical, temperature, chemical, toxic or pharmaceutical stress. One example is hypoxia, defined herein as a physiological state in which oxygen demand by the cell exceeds its supply to the cell. The transcriptome or proteome under this set of experimental conditions may be compared to the transcriptome or proteome under conditions of normoxia, when oxygen supply is in concordance with the demand by the cell.

The transcriptomes or proteomes may also be compared under different genetic conditions. By "genetic conditions" is meant that the genotype of the compared cell populations contains a different genetic component. This may be the presence of one or more different, non-endogenous nucleic acid molecules in

the cell, herein referred to collectively as "genetic elements". Such genetic element(s) may potentially be incorporated into the genome of the cell, or alternatively may exist as a separate genetic entity, for example, as an extra-chromosomal element such as a plasmid or episome. Alternatively, the genome may have been perturbed by external intervention, for example, to increase or decrease the expression of a particular gene or genes. A number of variations on this theme are possible, including the overexpression of a genetic element via the administration of the functional gene, the overexpression of a genetic element via the administration of a regulator of the functional gene (such as, for example, a transcription factor [either natural or artificially constructed via the fusion of a DNA binding domain with an activator domain]), the inhibition of the expression of a functional gene (for example, using antisense RNA or ribozymes), the inhibition of the expression of a functional gene (for example, using a transdominant protein) and the inhibition of the expression of a functional gene (for example, using a repressor protein that is either natural or artificially constructed from a DNA binding protein fused to a repressor domain).

A particular example of a genetic perturbation as envisaged herein, that forms one preferred embodiment of the method of the present invention, is the so-called "Smartomics" technology that forms the basis for co-pending, co-owned International patent application PCT/GB01/00758. According to this technology, a heterologous nucleic acid is introduced into a primary cell to augment a specific natural physiological response. "Smartomics" may be applied to the current invention by measuring and comparing cellular responses to a heterologous gene in two or more distinct cell types, both with and without the natural physiological stimulus. Lentivirus technology is used to introduce the heterologous nucleic acid molecule in such a way that there is negligible perturbation of endogenous gene expression. For this reason, this technology exhibits significant benefits over conventional technology of a similar nature, since the prior art methods are generally invasive, having downstream effects other than the simple introduction of the heterologous nucleic acid molecule. The Smartomics technology allows much more precise measurements to be taken of the effect of introducing the heterologous nucleic acid.

The method of the invention allows the identification of genes that are implicated in a specific disease or physiological condition. The genes identified in this way are candidate targets for antagonists or agonists that modulate disease states pertinent to that specialised cell type. This allows the development of selective agonists and antagonists, rather than broad spectrum agonists and antagonists. This approach thus adds value in the selective treatment of disease. Furthermore the identified genes are associated with regulatory elements that provide alternative and additional candidate targets for exploitation for the delivery of gene products to that cell in a cell-specific fashion. The genes and regulatory elements identified according to the method of the invention can be used directly in therapeutic applications via gene therapy, via recombinant protein methods or via chemical mimetics or as targets for the

development of agonists and antagonists such as antibodies, small chemical molecules, peptides, regulatory nucleic acids.

The step of comparison of the transcriptomes or proteomes of the first and second specialised cell types under first and second experimental conditions may be effected using any approach that allows the quantitative comparison of gene or protein expression, and a number of such means will be known to those of skill in the art. Such experiments have only become possible in recent years, due to certain advances in technology that have allowed the large scale, high throughput analysis of gene expression.

One example of a method that allows the comparison of the transcriptome of a specific cell type with a second or subsequent transcriptome involves the generation of a set of clones that represent all the transcripts expressed in a cell under the conditions in which the cell is maintained. This may be done by constructing a cDNA library, in which copies of all mRNA transcripts expressed in the cell are cloned into a suitable vector for subsequent analysis.

Such libraries may be normalised cDNA libraries, in which the distribution of genes in the library has been biased to reduce the number of clones that represent genes with large numbers of transcripts (such as, for example, beta-actin) and thus reduce the repetitive nature of the library. Normalisation thus acts to reduce the frequency of genes expressed at high levels and to enhance the frequency of genes expressed at low levels (see de Fatima Bonaldo *et al.*, Genome Research 6: 791-806 (1996)).

Libraries may also be subtracted cDNA libraries, in which the distribution of genes is manipulated to remove genes that are expressed in both mRNA populations used to construct the library. The commercially-available PCR Select kit (Clontech, Inc) is an example of a system useful to generate such libraries.

cDNA clones generated as reflective of the transcriptome of a specific cell type may then be amplified, and processed to evaluate the identity of the nucleic acid clones. For example, multiple clones may be picked and used as template for PCR amplification. The PCR products may then be arrayed onto membranes or glass slides to create nucleic acid arrays. For expression profiling, these arrays are then hybridised to complex nucleic acid probes in order to quantitate the abundance of individual genes contained in the probes.

A recent summary of nucleic acid array technology that is useful in the analysis of the transcriptome of a cell population is provided in Nature Genetics, (1999) (21 suppl; 1-61). There are various types of array technology currently used, including "microarrays", or "chips", which are high density cDNA arrays produced on glass slides, often produced using photolithography. A second type of array is the

"macroarray", which is an array with sub-millimetre spot-spot distances produced on a nylon membrane. One example of this type of array are the nylon-based microarrays sold commercially by Research Genetics Inc. (termed Research Genetics Human GeneFilters) that each contain 5,300 cDNA fragments of known identity. The whole series of arrays covers some 35,000 cDNA fragments. This particular array system (and others like it) allow the identification of transcripts that are down-regulated, as well as those that are up-regulated, since the range of genes used to manufacture the arrays are not biased.

The step of comparison may be effected by utilising subtracted cDNA libraries. Using this approach, the transcriptome of one specialised cell type under first experimental conditions is subtracted against the transcriptome under second experimental conditions. This reveals the differences in expression under the two experimental conditions tested. When this is performed for both specialised cell types, the differential regulation of gene expression under the two experimental conditions is revealed.

The step of comparison is through the detection of genes that are differentially regulated in the two specialised cell types examined under the first and second experimental conditions. As an example, a human cardiomyoblast (cell type A) and a human macrophage (cell type B) may be placed at the same temperature and at a high oxygen tension (first experimental conditions [1]). Cells from the same cell types are also incubated at this temperature, yet under conditions of low oxygen tension (second experimental conditions [2]). In this simple example, there are then a minimum of four combinations of cell type and condition, A[1], B[1], A[2] and B[2]. "Snapshots" are taken of the transcriptomes of both cell types under the "normoxic" and the "hypoxic" experimental conditions, by preparing messenger RNA from all four combinations. Differences in the regulation of genes can then be analysed, for example, using a process of subtractive hybridisation.

The mechanism of transcriptome comparison in the above example may be as follows. Subtracted cDNA libraries are separately prepared for hypoxic macrophages and cardiomyoblasts; for both cell types, their cDNA under normoxic conditions is subtracted against their cDNA under hypoxic conditions. This might be effected by harvesting RNA from cells both in normoxia and hypoxia, and preparing cDNA. Subtractive hybridization, optionally including suppression PCR, may then be performed to remove genes from the hypoxic cell cDNA which are also present in cDNA from normoxic cells. Insert DNA from these subtracted libraries can then be amplified and arrayed onto duplicate membranes. Quantitative hybridization with pre-library cDNA material (normoxia and hypoxia) then allows the comparison of differentially-expressed clones in the two cell types. The clones representing hypoxia-inducible genes may be then be identified, for example, by sequencing.

Other techniques that are suitable for the analysis of the transcriptome of a specific cell type include serial analysis of gene expression (SAGE; Velculescu *et al.*, Science (1995) 270; 484-487), Selective amplification via biotin- and restriction-mediated enrichment (SABRE) (Lavery *et al*, (1997), *PNAS USA* 94: p6831-6836); Differential display (for example, indexing differential display reverse transcriptase polymerase chain reaction (DDRT-PCR; Mahadeva *et al.* (1998) J. Mol.Biol. 284, 1391-1398)); representational difference analysis (RDA) (Hubank (1999) Methods in Enzymology 303: 325-349); differential screening of cDNA libraries (see Sagerstrom *et al.* (1997) Annu. Rev. Biochem. 66: 751-783); "Advanced Molecular Biology", R.M. Twyman (1998) Bios Scientific Publishers, Oxford; "Nucleic Acid Hybridization", M. L. M. Anderson (1999) Bios Scientific Publishers, Oxford); Northern blotting; RNAse protection assays; S1-nuclease protection assays; RT-PCR; real time RT-PCR (Taq-man); EST sequencing; massively parallel signature sequencing (MPSS); and sequencing by hybridisation (SBH) (see Drmanac R. *et al* (1999), Methods in Enzymology 303:165-178). Many of these techniques are reviewed in "Comparative gene-expression analysis" Trends Biotechnol. 1999 Feb;17(2):73-8.

Methods such as these have been applied widely to study mechanisms of biological response. In particular, microarrays have been used widely to compare gene expression levels between normal and diseased tissue. More typically, however, comparisons are performed to detect changes in gene expression that are associated with specific aspects of disease progression or pathology. For instance, a study of prostate cancer would examine changes associated with the step-wise progression to full malignancy or the dependence on androgens for growth.

Transcriptome analysis is complemented by the analysis of the complete protein make-up of a cell, referred to as proteomics. The use of two dimensional SDS-PAGE gels in combination with amino acid sequencing by mass spectrometry is currently the most widely-used technique in this field (see "Proteomics to study genes and genomes" Akhilesh Pandey and Matthias Mann, (2000), Nature 405: 837-846). Additionally, the recent developments in the field of protein and antibody arrays now allow the simultaneous detection of a large number of proteins. For example, low-density protein arrays on filter membranes, such as the universal protein array system (Ge H, (2000) Nucleic Acids Res. 28(2), e3) allow imaging of arrayed antigens using standard ELISA techniques and a scanning charge-coupled device (CCD) detector. Immuno-sensor arrays have also been developed that enable the simultaneous detection of clinical analytes. It is now possible using protein arrays, to profile protein expression in bodily fluids, such as in sera of healthy or diseased subjects, as well as in patients pre- and post-drug treatment.

Antibody arrays also facilitate the extensive parallel analysis of numerous proteins that are hypothetically implicated in a disease or particular physiological state. A number of methods for the preparation of antibody arrays have recently been reported (see Cahill, Trends in Biotechnology, 2000 7:47-51).

It is not the intention here to review studies that have been conducted in this area previously. However, one example of a physiological condition that has already received considerable attention is the response to hypoxia. Several patent applications have now been published that involve an examination of the genetic response to hypoxia (see WO00/12139, Quark Biotech, Inc.; WO00/12525, Quark Biotech, Inc.; WO99/09049, Quark Biotech, Inc.; WO99/09046, Quark Biotech, Inc.; WO99/48916, The Board of Trustees of the Leland Stanford Jr. University). These patent applications generally utilise methods of subtractive hybridisation and differential expression gene microarray analysis to examine this genetic response in certain cell lines. The studies have implicated specific genes as being either repressed or induced under hypoxic conditions as compared to their expression under normoxic conditions. These genes are taught as being useful generally in all cell types, being involved in the (generic) hypoxic response.

Significantly, the present invention extends this work, and, indeed, defines a significant advance over similar work that has been performed on the genetic mechanisms that act in response to other physiological or genetic stimuli. The present inventors, using the novel methods disclosed herein, have discovered that far from being generic, the cellular response to many physiological conditions differs markedly between different cell types. The cellular response that has been studied in order to illustrate this finding is the response to hypoxia. From these results, it has been inferred herein, quite reasonably, that far from being generic, cellular response mechanisms differ widely, depending on cell type.

This discovery has far-reaching implications as regards the design of therapeutic agents that are effective to counter a disease or physiological condition. For example, an agent that is effective to prevent the drastic effects of hypoxia in a neurone (the effects of which include stroke) might be totally ineffective in countering the same effects in a cardiomyocyte (chronic ischemic heart disease). Through analysing the mechanism of the hypoxic response in different cell types, it may be, in contrast to the example given above, that a particular gene is involved in the hypoxic response in both cardiomyocytes and neurones. Were this to be the case, this would allow the design of a combined medicament, for example, a combined cardioprotective and neuroprotective agent. There thus remains a great need for the identification of proteins implicated in the physiological mechanism of hypoxia.

According to a further aspect of the invention, there are provided genes and proteins that are identified using a method according to any one of the above-described aspects of the invention. Certain proteins, whose sequences are identified herein as SEQ ID Nos: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 63, 67, 69, 73, 75, 77, 85, 87, 89, 91, 93, 95, 99, 103, 113, 115, 119, 121, 129, 131, 133, 137, 139, 141, 145, 151, 153, 157, 159, 163, 169, 181, 187, 201, 205, 207 and 209, are functionally annotated for the first time. At present, all of these sequences are only

identified as "hypothetical proteins" in the public databases. Each and every one of these sequences forms an embodiment of this aspect of the invention.

The invention also includes proteins whose amino acid sequences are encoded by a nucleic acid sequence recited in various cDNAs and ESTs deposited in the public databases, or encoded by a gene identified from such an EST. These cDNAs and ESTs are presented herein as SEQ ID Nos: 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 92a, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214 and 216. At present, all of these cDNA and EST sequences are functionally unannotated in the public databases. Each and every one of these sequences forms an embodiment of this aspect of the invention.

One embodiment of this aspect of the invention provides substantially purified polypeptide, which polypeptide:

i) comprises the amino acid sequence as recited in any one of SEQ ID Nos: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 63, 67, 69, 73, 75, 77, 85, 87, 89, 91, 93, 95, 99, 103, 113, 115, 119, 121, 129, 131, 133, 137, 139, 141, 145, 151, 153, 157, 159, 163, 169, 181, 187, 201, 205, 207 or 209;

ii) has an amino acid sequence encoded by a nucleic acid sequence recited in any one of SEQ ID Nos: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 92a, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214 and 216 or encoded by a gene identified from an EST recited in any one of these SEQ ID Nos;

iii) is a fragment of a polypeptide according to i) or ii), provided that said fragment retains a biological activity possessed by the full length polypeptide of i) or ii), or has an antigenic determinant in common with the polypeptide of i) or ii); or

iv) is a functional equivalent of a polypeptide of i), ii) or (iii).

The polypeptide sequences recited in SEQ ID Nos: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 63, 67, 69, 73, 75, 77, 85, 87, 89, 91, 93, 95, 99, 103, 113, 115, 119, 121, 129, 131, 133, 137, 139, 141, 145, 151, 153, 157, 159, 163, 169, 181, 187, 201, 205, 207 and 209 were, prior to the present disclosure, unannotated in the literature and public sequence

databases. Accordingly, until now, no biological function has been attributed to these polypeptide sequences; each of these sequences is generally labelled in the databases as a "hypothetical protein". The methods of the present invention, described above, have now elucidated a biological function for these polypeptides, in that they have been found to be differentially regulated under physiological conditions of hypoxia.

These discoveries allow the development of regulators, such as small drug molecules, that affect the activity of these polypeptides, so allowing diseases and physiological conditions that are caused by hypoxia, or in which hypoxia has been implicated, to be treated. These discoveries also allow the development of diagnostic agents that are suitable for the detection of hypoxia in biological tissues and, through the identification of mutations and polymorphisms (such as SNPs) within genes coding for the proteins implicated herein, allows the assessment of an individual's risk of being susceptible to diseases and physiological conditions in which hypoxia is implicated.

The biological activity of polypeptides whose sequences are listed in SEQ ID Nos: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 63, 67, 69, 73, 75, 77, 85, 87, 89, 91, 93, 95, 99, 103, 113, 115, 119, 121, 129, 131, 133, 137, 139, 141, 145, 151, 153, 157, 159, 163, 169, 181, 187, 201, 205, 207 and 209 has been found to be hypoxia-regulated. The expression of some of these polypeptides has been found to be induced under conditions of hypoxia, whilst the expression of other polypeptides has been found to be repressed. By "hypoxia-induced" is meant that the polypeptide is expressed at a higher level when a cell is exposed to hypoxic conditions as compared to its expression level under normoxic conditions. By "hypoxia-repressed" is meant that the polypeptide is expressed at a lower level when a cell is exposed to hypoxic conditions as compared to its expression level under normoxic conditions.

The following polypeptides have been found to be hypoxia-induced: those polypeptides whose amino acid sequence is recited in SEQ ID Nos: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 63, 67, 69, 73, 75, 77, 85, 87, 89, 91, 93, 95, 99, 103, 113, 115, 119, 121, 129, 131, 133, 137, 139 and 141; and those polypeptides whose amino acid sequence is encoded by a nucleic acid sequence recited in SEQ ID Nos.: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 92a, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142 and 144 or is encoded by a gene identified from an EST recited in any one of these SEQ ID Nos..

The following polypeptides have been found to be hypoxia-repressed: those polypeptides whose amino acid sequence is recited in SEQ ID Nos.: 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 145, 151, 153, 157, 159, 163, 169, 181, 187, 201, 205, 207 and 209; and those polypeptides whose amino acid sequence

is encoded by a nucleic acid sequence recited in SEQ ID Nos.: 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214 and 216, or encoded by a gene identified from an EST recited in any one of these SEQ ID Nos.

For the purposes of this document, the term "hypoxia" should be taken to mean an environment of oxygen tension such that the oxygen content is between about 5% and 0.1% (v/v). In most cases, hypoxic tissue will have an oxygen content that is less than or equal to about 2%. The term "normoxia" should be taken to mean conditions comprising a normal level of oxygen for the environment concerned. Normoxic tissue typically has an oxygen content above about 5%.

The polypeptide sequences whose amino acid sequence is encoded by a nucleic acid sequence recited in SEQ ID Nos: 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 92a, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214 and 216, or whose amino acid sequence is encoded by a gene identified from an EST recited in any one of these SEQ ID Nos., were also, prior to the present disclosure, unannotated in the literature and public sequence databases, meaning that until now, no biological function has been attributed to these polypeptide sequences.

The sequences in this group fall into a number of different categories. The first of these are cDNA clones, for which a protein sequence has not been predicted by the depositor. A second category is expressed sequence tag (EST) sequences that are represented in the UniGene database (http://www.ncbi.nlm.nih.gov/UniGene/), which contain modest or weak homology to known proteins when translated. ESTs are single-pass sequence files of the 5' region of an organism's expressed genome as accessed via a force cloned cDNA library. EST sequences tend to be short and as a general rule are error-prone. UniGene (see http://www.ncbi.nlm.nih.gov/Web/Newsltr/aug96.html for review) is an experimental system for automatically partitioning these EST sequences into a non-redundant set of gene-oriented clusters. Each UniGene cluster contains sequences that represent a unique gene, as well as related information such as the tissue types in which the gene has been expressed and map location. A third category of hits identified by the methods described herein is EST sequences that are contained in Unigene clusters, but which are not annotated and exhibit no homologies to proteins contained in the public databases. The fourth and final category encompasses singleton EST sequence entries that are not incorporated as entries in the Unigene database and that only appear as single entries in the public databases.

The methods of the present invention, described above, have now elucidated a biological function for polypeptides that are encoded by genes incorporating cDNA and EST sequences that fall into the four categories set out above, in that these sequences have been found to be differentially regulated under physiological conditions of hypoxia. Such polypeptides may have an amino acid sequence that is encoded by a nucleic acid sequence recited in any one of SEQ ID Nos: 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 92a, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214 and 216. However, the EST sequences in particular may not be part of the actual coding sequence for a gene, often representing regulatory regions of the gene, or regions that are transcribed, but not translated into polypeptide. Accordingly, this aspect of the invention also includes polypeptides that are encoded by a gene identified from an EST recited in any one of SEQ ID Nos: 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 92a, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214 and 216;

Polypeptides of this aspect of the invention are intended to include fragments of polypeptides according to i) or ii) as defined above, provided that the fragment retains a biological activity that is possessed by the full length polypeptide of i) or ii), or has an antigenic determinant in common with the polypeptide of i) or ii). As used herein, the term "fragment" refers to a polypeptide having an amino acid sequence that is the same as part, but not all, of an amino acid sequence as recited in any one of SEQ ID Nos: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 63, 67, 69, 73, 75, 77, 85, 87, 89, 91, 93, 95, 99, 103, 113, 115, 119, 121, 129, 131, 133, 137, 139, 141, 145, 151, 153, 157, 159, 163, 169, 181, 187, 201, 205, 207 and 209, an amino acid sequence that is encoded by a nucleic acid sequence recited in any one of SEQ ID Nos. 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 92a, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214 and 216, or an amino acid sequence that is encoded by a gene that is linked to a nucleic acid sequence recited in any one of these SEQ ID Nos. The fragments should comprise at least *n* consecutive amino acids from the sequence and, depending on the particular sequence, *n* preferably is 7 or more (for example, 8, 10, 12, 14, 16, 18, 20 or more). Small fragments may form an antigenic determinant.

Such fragments may be isolated fragments, that are not part of or fused to other amino acids or polypeptides, or they may be comprised within a larger polypeptide, of which they form a part or region.

When comprised within a larger polypeptide, a fragment of the invention most preferably forms a single continuous region. For instance, certain preferred embodiments relate to a fragment having a pre – and/or pro- polypeptide region fused to the amino terminus of the fragment and/or an additional region fused to the carboxyl terminus of the fragment. However, several fragments may be comprised within a single larger polypeptide.

The polypeptides of the present invention or their immunogenic fragments (comprising at least one antigenic determinant) can be used to generate ligands, such as polyclonal or monoclonal antibodies, that are immunospecific for the polypeptides. Such antibodies may be employed to isolate or to identify clones that express a polypeptide according to the invention or, for example, to purify the polypeptide by affinity chromatography. Such antibodies may also be employed as diagnostic or therapeutic aids, amongst other applications, as will be apparent to the skilled reader.

The term "immunospecific" means that an antibody has substantially greater affinity for a polypeptide according to the invention than their affinity for related polypeptides. As used herein, the term "antibody" is intended to include intact molecules as well as fragments thereof, such as Fab, $F(ab')_2$ and scFv, which are capable of binding to the antigenic determinant in question.

The invention also includes functional equivalents of a polypeptide of i), ii) or (iii) as recited above. A functionally-equivalent polypeptide according to this aspect of the invention may be a polypeptides that is homologous to a polypeptide whose sequence is explicitly recited herein. Two polypeptides are said to be "homologous" if the sequence of one of the polypeptides has a high enough degree of identity or similarity to the sequence of the other polypeptide for the skilled person to determine that they are similar in origin and function. Preferably, homology is used to refer to sequence identity. "Identity" indicates that at any particular position in the aligned sequences, the amino acid residue is identical between the sequences. "Similarity" indicates that, at any particular position in the aligned sequences, the amino acid residue is of a similar type between the sequences. Degrees of identity and similarity can be readily calculated according to methods known in the art (see, for example, Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing. Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York, 1993). Advantageously, the BLAST algorithm is employed, with parameters set to default values. The BLAST algorithm is described in detail at http://www.ncbi.nih.gov/BLAST/blast_help.html, which is incorporated herein by reference. The search parameters are defined as follows, and are advantageously set to the defined default parameters.

Advantageously, "substantial homology" when assessed by BLAST equates to sequences which match with an EXPECT value of at least about 7, preferably at least about 9 and most preferably 10 or more. The default threshold for EXPECT in BLAST searching is usually 10.

BLAST (Basic Local Alignment Search Tool) is the heuristic search algorithm employed by the programs blastp, blastn, blastx, tblastn, and tblastx; these programs ascribe significance to their findings using the statistical methods of Karlin and Altschul (see http://www.ncbi.nih.gov/BLAST/blast_help.html) with a few enhancements. The BLAST programs were tailored for sequence similarity searching, for example to identify homologues to a query sequence. The programs are not generally useful for motif-style searching. For a discussion of basic issues in similarity searching of sequence databases, see Altschul et al. (1994) Nature Genetics 6:119-129.

The five BLAST programs available at http://www.ncbi.nlm.nih.gov perform the following tasks:

blastp compares an amino acid query sequence against a protein sequence database;

blastn compares a nucleotide query sequence against a nucleotide sequence database;

blastx compares the six-frame conceptual translation products of a nucleotide query sequence (both strands) against a protein sequence database;

tblastn compares a protein query sequence against a nucleotide sequence database dynamically translated in all six reading frames (both strands).

tblastx compares the six-frame translations of a nucleotide query sequence against the six-frame translations of a nucleotide sequence database.

BLAST uses the following search parameters:

HISTOGRAM Display a histogram of scores for each search; default is yes. (See parameter H in the BLAST Manual).

DESCRIPTIONS Restricts the number of short descriptions of matching sequences reported to the number specified; default limit is 100 descriptions. (See parameter V in the manual page). See also EXPECT and CUTOFF.

ALIGNMENTS Restricts database sequences to the number specified for which high-scoring segment pairs (HSPs) are reported; the default limit is 50. If more database sequences than this happen to satisfy the statistical significance threshold for reporting (see EXPECT and CUTOFF below), only the matches ascribed the greatest statistical significance are reported. (See parameter B in the BLAST Manual).

EXPECT The statistical significance threshold for reporting matches against database sequences; the default value is 10, such that 10 matches are expected to be found merely by chance, according to the stochastic model of Karlin and Altschul (1990). If the statistical significance ascribed to a match is greater than the EXPECT threshold, the match will not be reported. Lower EXPECT thresholds are more

stringent, leading to fewer chance matches being reported. Fractional values are acceptable. (See parameter E in the BLAST Manual).

CUTOFF Cutoff score for reporting high-scoring segment pairs. The default value is calculated from the EXPECT value (see above). HSPs are reported for a database sequence only if the statistical significance ascribed to them is at least as high as would be ascribed to a lone HSP having a score equal to the CUTOFF value. Higher CUTOFF values are more stringent, leading to fewer chance matches being reported. (See parameter S in the BLAST Manual). Typically, significance thresholds can be more intuitively managed using EXPECT.

MATRIX Specify an alternate scoring matrix for BLASTP, BLASTX, TBLASTN and TBLASTX. The default matrix is BLOSUM62 (Henikoff & Henikoff, 1992). The valid alternative choices include: PAM40, PAM120, PAM250 and IDENTITY. No alternate scoring matrices are available for BLASTN; specifying the MATRIX directive in BLASTN requests returns an error response.

STRAND Restrict a TBLASTN search to just the top or bottom strand of the database sequences; or restrict a BLASTN, BLASTX or TBLASTX search to just reading frames on the top or bottom strand of the query sequence.

FILTER Mask off segments of the query sequence that have low compositional complexity, as determined by the SEG program of Wootton & Federhen (1993) Computers and Chemistry 17:149-163, or segments consisting of short-periodicity internal repeats, as determined by the XNU program of Claverie & States (1993) Computers and Chemistry 17:191-201, or, for BLASTN, by the DUST program of Tatusov and Lipman (see http://www.ncbi.nlm.nih.gov). Filtering can eliminate statistically significant but biologically uninteresting reports from the blast output (e.g., hits against common acidic-, basic- or proline-rich regions), leaving the more biologically interesting regions of the query sequence available for specific matching against database sequences.

Low complexity sequence found by a filter program is substituted using the letter "N" in nucleotide sequence (e.g., "NNNNNNNNNNNNN") and the letter "X" in protein sequences (e.g., "XXXXXXXXX").

Filtering is only applied to the query sequence (or its translation products), not to database sequences. Default filtering is DUST for BLASTN, SEG for other programs.

It is not unusual for nothing at all to be masked by SEG, XNU, or both, when applied to sequences in SWISS-PROT, so filtering should not be expected to always yield an effect. Furthermore, in some cases, sequences are masked in their entirety, indicating that the statistical significance of any matches reported against the unfiltered query sequence should be suspect.

NCBI-gi Causes NCBI gi identifiers to be shown in the output, in addition to the accession and/or locus name.

Most preferably, sequence comparisons are conducted using the simple BLAST search algorithm provided at http://www.ncbi.nlm.nih.gov/BLAST.

Alternatively, sequence homology may be determined by algorithms such as FastA, available at http://biology.ncsa.uiuc.edu/BW30/BW.cgi. FastA is considered to be superior to BLAST for alignment of short sequences. Advantageously, the FastA algorithm is employed using default parameters at http://biology.ncsa.uiuc.edu/BW30/BW.cgi.

Typically, greater than 50% identity between two polypeptides is considered to be an indication of functional equivalence, provided that either the biological activity of the polypeptide is retained or the polypeptides possess an antigenic determinant in common. Preferably, a functionally equivalent polypeptide according to this aspect of the invention exhibits a degree of sequence identity with a polypeptide sequence explicitly identified herein, or with a fragment thereof, of greater than 50%. More preferred polypeptides have degrees of identity of greater than 60%, 70%, 80%, 90%, 95%, 98% or 99%, respectively.

Functionally-equivalent polypeptides according to the invention are therefore intended to include natural biological variants (for example, allelic variants or geographical variations within the species from which the polypeptides are derived) and mutants (such as mutants containing amino acid substitutions, insertions or deletions) of the polypeptides whose sequences are explicitly recited herein. Such mutants may include polypeptides in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; among the basic residues Lys and Arg; or among the aromatic residues Phe and Tyr.

Particularly preferred are variants in which several, i.e. between 5 and 10, 1 and 5, 1 and 3, 1 and 2 or just 1 amino acids are substituted, deleted or added in any combination. Especially preferred are silent substitutions, additions and deletions, which do not alter the properties and activities of the protein. Also especially preferred in this regard are conservative substitutions. "Mutant" polypeptides also include polypeptides in which one or more of the amino acid residues include a substituent group.

As discussed above, using a method according to the above-described aspects of the invention it has now been discovered, most surprisingly, that the response to hypoxia differs between different specialised cell types or between different physiological states of the same cell type. For example, it has been found that in macrophage cells, different polypeptides are induced/repressed during different physiological states.

Furthermore, it has been found that a subset of this group of polypeptides are regulated only in activated macrophage cells. Macrophages possess various biological activities, including cytotoxic effects towards tumour cells and phagocytosis of bacteria or cellular debris. These form an important and potent arm of innate immunity, and as such must be finely regulated. In the absence of interactions with pathogens or other immune cells, the aforementioned activities of the macrophage are greatly reduced (i.e. resting macrophages). When given appropriate stimuli, such as contact with the lipopolysaccharide surface of bacteria, and/or exposure to T-cell derived interferon gamma, the functional activities of the macrophage are greatly potentiated (i.e. activated macrophage).

The expression of a further subset of these polypeptides has been found herein to be induced in activated macrophages under conditions of hypoxia, whilst a still further subset has been found herein to be repressed in activated macrophages under conditions of hypoxia.

In resting macrophage cells, it has been found that different polypeptides are induced/repressed during the biological response to hypoxia. For example, it has been found that a subset of this group of polypeptides are regulated only in resting macrophage cells. The expression of a further subset of these polypeptides has been found herein to be induced in resting macrophages under conditions of hypoxia, whilst a still further subset has been found herein to be repressed in resting macrophages under conditions of hypoxia.

According to a further aspect of the invention, there is provided a purified and isolated nucleic acid molecule that encodes a polypeptide according to any one of the aspects of the invention discussed above. Such a nucleic acid molecule may consist of the nucleic acid sequence as recited in any one of SEQ ID Nos. 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 92a, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214 and 216, or form a redundant equivalent or fragment thereof. This aspect of the invention also includes a purified nucleic acid molecule which hydridizes under high stringency conditions with a nucleic acid molecule as described above.

According to a further aspect of the invention, there is provided an expression vector that contains a purified and isolated nucleic acid molecule according to the aspects of the invention described above. The invention also incorporates a delivery vehicle, such as a liposome, comprising a nucleic acid according to the above-described aspects of the invention.

In a further aspect, the invention provides a host cell transformed with a vector of the above-described aspect of the invention.

In a still further aspect, the invention provides a ligand that binds specifically to a polypeptide according to the above-described aspects of the invention. The ligand may be an antagonist ligand that inhibits the biological activity of the polypeptide, or may be an agonist ligand that activates the hypoxia-induced activity of the polypeptide to augment or potentiate a hypoxia-induced activity.

In a still further aspect of the invention, there is provided a ligand which binds specifically to, and which preferably inhibits the hypoxia-induced activity of, a polypeptide according to any one of the above-described aspects of the invention. Such a ligand may, for example, be an antibody that is immunospecific for the polypeptide in question.

According to a further aspect, the invention provides a polypeptide, a nucleic acid molecule, vector or ligand as described above, for use in therapy or diagnosis of a disease or abnormal physiological condition. Preferably, the disease or abnormal physiological condition that is affected by hypoxia; examples of such diseases include cancer, ischaemic conditions (such as stroke, coronary arterial disease, peripheral arterial disease), reperfusion injury, retinopathy, neonatal stress, preeclapmsia, atherosclerosis, inflammatory conditions (including rheumatoid arthritis), hair loss and wound healing. The undesired celluar process involved in said diseases might include, but is not restricted to; tumorigenesis, angiogenesis, apoptosis, inflammation or erythropoiesis. The undesired biochemical processes involved in said cellular processes might include, but is not restricted to, glycolysis, gluconeogenesis, glucose transportation, catecholamine synthesis, iron transport or nitric oxide synthesis.

According to the invention, a number of known proteins have also been implicated in the biological response to hypoxia. The functions of these proteins are known, meaning that these functions have been annotated in the public databases. The sequences of these proteins are presented in SEQ ID Nos.: 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 385, 387, 389, 391, 393, 395, 397, 399, 401, 403, 405, 407, 409, 411, 413, 415, 417, 419, 421, 423, 425, 427, 429, 431, 433, 435, 437, 439, 441, 443, 445, 447, 449, 451, 453, 455, 457, 459, 461, 463, 465, 467, 469, 471, 473, 475, 477, 479, 481, 483, 485 and 487.

According to a further aspect of the invention, there is provided a substantially purified polypeptide, which polypeptide:

i) comprises the amino acid sequence as recited in any one of SEQ ID Nos: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 63, 67, 69, 73, 75, 77, 85, 87, 89, 91, 93, 95, 99, 103, 113, 115, 119, 121, 129,

131, 133, 137, 139, 141, 145, 151, 153, 157, 159, 163, 169, 181, 187, 201, 205, 207 or 209 or any one of SEQ ID Nos.: 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 385, 387, 389, 391, 393, 395, 397, 399, 401, 403, 405, 407, 409, 411, 413, 415, 417, 419, 421, 423, 425, 427, 429, 431, 433, 435, 437, 439, 441, 443, 445, 447, 449, 451, 453, 455, 457, 459, 461, 463, 465, 467, 469, 471, 473, 475, 477, 479, 481, 483, 485, 487, 489 and 491;

ii) has an amino acid sequence encoded by a nucleic acid sequence recited in any one of SEQ ID Nos: 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 92a, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214 and 216, or encoded by a gene identified from an EST recited in any one of these SEQ ID Nos;

iii) is a fragment of a polypeptide according to i) or ii), provided that said fragment retains a biological activity possessed by the full length polypeptide of i) or ii), or has an antigenic determinant in common with the polypeptide of i) or ii); or

iv) is a functional equivalent of a polypeptide of i), ii) or (iii);

for use in the diagnosis or therapy of tumourigenesis, angiogenesis, apoptosis, the biological response to hypoxia conditions, or a hypoxic-associated pathology.

The invention also provides a purified and isolated nucleic acid molecule that encodes a polypeptide according to this aspect of the invention, for use in the diagnosis or therapy of tumourigenesis, angiogenesis, apoptosis, the biological response to hypoxia conditions, or a hypoxic-associated pathology. The sequences of these molecules are provided in SEQ ID Nos.: 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 322, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458, 460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484,

486 and 488. As described above for the EST nucleic acid sequences annotated herein, this aspect of the invention includes redundant equivalents and fragments of the sequences explicitly recited in SEQ ID Nos.: 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 322, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458, 460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486 and 488, and purified nucleic acid molecules which hybridize under high stringency conditions with such nucleic acid molecules, and vectors containing such nucleic acid molecules for use in the diagnosis or therapy of tumourigenesis, angiogenesis, apoptosis, the biological response to hypoxia conditions, or a hypoxic-associated pathology.

This aspect of the invention also includes ligands which bind specifically to, and which preferably inhibit the hypoxia-induced activity of, a polypeptide listed in SEQ ID Nos.: 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 385, 387, 389, 391, 393, 395, 397, 399, 401, 403, 405, 407, 409, 411, 413, 415, 417, 419, 421, 423, 425, 427, 429, 431, 433, 435, 437, 439, 441, 443, 445, 447, 449, 451, 453, 455, 457, 459, 461, 463, 465, 467, 469, 471, 473, 475, 477, 479, 481, 483, 485 and 487, for use in the diagnosis or therapy of tumourigenesis, angiogenesis, apoptosis, the biological response to hypoxia conditions, or a hypoxic-associated pathology.

The invention also provides a pharmaceutical composition suitable for modulating hypoxia and/or ischaemia, comprising a therapeutically-effective amount of a a polypeptide, a nucleic acid molecule, vector or ligand as described above, in conjunction with a pharmaceutically-acceptable carrier.

The invention also provides a vaccine composition comprising a polypeptide, or a nucleic acid molecule as described above.

The invention also provides a method of treating a disease in a patient in need of such treatment by administering to a patient a therapeutically effective amount of a polypeptide, a nucleic acid molecule, vector, ligand or pharmaceutical composition as described above. For diseases in which the expression of the natural gene or the activity of the polypeptide is lower in a diseased patient when compared to the level of expression or activity in a healthy patient, the polypeptide, nucleic acid molecule, ligand, compound or composition administered to the patient should be an agonist. For diseases in which the

expression of the natural gene or activity of the polypeptide is higher in a diseased patient when compared to the level of expression or activity in a healthy patient, the polypeptide, nucleic acid molecule, vector, ligand, compound or composition administered to the patient is an antagonist. By the term "agonist" is meant herein, any polypeptide, peptide, synthetic molecule or organic molecule that functions as an activator, by increasing the effective biological activity of a polypeptide, for example, by increasing gene expression or enzymatic activity. By the term "antagonist" is meant herein, any polypeptide, peptide, synthetic molecule or organic molecule that functions as an inhibitor, by decreasing the effective biological activity of the gene product, for example, by inhibiting gene expression of an enzyme or a pharmacological receptor.

The invention also provides for the use of a polypeptide, nucleic acid molecule, vector, ligand or pharmaceutical composition according to any one of the above-described aspects of the invention in modifying the response of a cell to conditions of hypoxia.

The invention also provides a polypeptide, nucleic acid molecule, vector, ligand or pharmaceutical composition according to any one of the above-described aspects of the invention, for use in the manufacture of a medicament for the treatment of a hypoxia-regulated condition.

The invention also provides a method of monitoring the therapeutic treatment of disease or physiological condition in a patient, comprising monitoring over a period of time the level of expression or activity of polypeptide, nucleic acid molecule, vector or ligand in tissue from said patient, wherein altering said level of expression or activity over the period of time towards a control level is indicative of regression of said disease or physiological condition.

The invention also provides a method of providing a hypoxia regulating gene, an apoptotic or an angiogenesis regulating gene by administering directly to a patient in need of such therapy an expressible vector comprising expression control sequences operably linked to one or more of the nucleic acid molecules as described above.

The invention also provides a method of diagnosing a hypoxia-regulated condition in a patient, comprising assessing the level of expression of a natural gene encoding a polypeptide according to any one of the aspects of the invention described above in tissue from said patient and comparing said level of expression or activity to a control level, wherein a level that is different to said control level is indicative of the hypoxia-related condition.

Such a method of diagnosis may be carried out *in vitro*. One example of a suitable method comprises the steps of: (a) contacting a ligand as described above with a biological sample under conditions suitable for the formation of a ligand-polypeptide complex; and (b) detecting said complex.

A further example of a suitable method may comprises the steps of: a) contacting a sample of tissue from the patient with a nucleic acid probe under stringent conditions that allow the formation of a hybrid complex between a nucleic acid molecule whose sequence is recited in any one of SEQ ID Nos.: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 92a, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 322, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458, 460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486 and 488, and the probe; b) contacting a control sample with said probe under the same conditions used in step a); and c) detecting the presence of hybrid complexes in said samples; wherein detection of levels of the hybrid complex in the patient sample that differ from levels of the hybrid complex in the control sample is indicative of the hypoxia-related condition.

A still further example of a suitable method may comprise the steps of: a) contacting a sample of nucleic acid from tissue of the patient with a nucleic acid primer under stringent conditions that allow the formation of a hybrid complex between a nucleic acid molecule whose sequence is recited in any one of SEQ ID Nos.: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 92a, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 322, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458, 460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486 and 488, and the primer; b) contacting a control sample with said primer under the same conditions used in step a); c) amplifying the sampled nucleic acid; and d) detecting the level of

amplified nucleic acid from both patient and control samples; wherein detection of levels of the amplified nucleic acid in the patient sample that differ significantly from levels of the amplified nucleic acid in the control sample is indicative of the hypoxia-related condition.

A still further example of a suitable method may comprised the steps of: a) obtaining a tissue sample from a patient being tested for the hypoxia-related condition; b) isolating a nucleic acid molecule according to any one of the above-described aspects of the invention from said tissue sample; and c) diagnosing the patient for the hypoxia-related condition by detecting the presence of a mutation which is associated with the hypoxia-related condition in the nucleic acid molecule as an indication of the hypoxia-related condition. This method may comprise the additional step of amplifying the nucleic acid molecule to form an amplified product and detecting the presence or absence of a mutation in the amplified product.

Particular hypoxia-related conditions that may be diagnosed in this fashion include cancer, ischaemia, reperfusion, retinopathy, neonatal stress, preeclapmsia, atherosclerosis, rheumatoid arthritis, undesired hair loss, cardiac arrest or stroke, for example, caused by a disorder of the cerebral, coronary or peripheral circulation.

In a further aspect, the invention provides a method for the identification of a compound that is effective in the treatment and/or diagnosis of a hypoxia-regulated condition, comprising contacting a polypeptide, nucleic acid molecule, or ligand according to any one of the above-described aspects of the invention with one or more compounds suspected of possessing binding affinity for said polypeptide, nucleic acid molecule or ligand, and selecting a compound that binds specifically to said nucleic acid molecule, polypeptide or ligand.

According to a still further aspect of the invention, there is provided a kit useful for diagnosing a hypoxia-regulated condition, comprising a first container containing a nucleic acid probe that hybridises under stringent conditions with a nucleic acid molecule according to any one of the aspects of the invention described above; a second container containing primers useful for amplifying said nucleic acid molecule; and instructions for using the probe and primers for facilitating the diagnosis of the hypoxia-regulated condition. The kit may additionally comprise a third container holding an agent for digesting unhybridised RNA.

To facilitate in the diagnosis of the hypoxia-regulated condition using one of the methods outlined above, in a further aspect, the invention provides an array of at least two nucleic acid molecules, wherein each of said nucleic acid molecules either corresponds to the sequence of, is complementary to the sequence of, or hybridises specifically to a nucleic acid molecule according to any one of the aspects of the invention described above. Such an array may contain nucleic acid molecules that either correspond to the sequence of, are complementary to the sequence of, or hybridise specifically to at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11,

12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 92a, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 215, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295 or more of the nucleic acid molecules implicated in a hypoxia-regulated condition as recited above. The nucleic acid molecules on the array may consist of oligonucleotides of between twelve and fifty nucleotides, more preferably, between forty and fifty nucleotides. Alternatively, the nucleic acid molecules on the array may consist of PCR-amplified cDNA inserts where the nucleic acid molecule is between 300-2000 nucleotides.

In a related aspect, again useful for diagnosis, the invention provides an array of antibodies, comprising at least two different antibody species, wherein each antibody species is immunospecific with a polypeptide implicated in a hypoxia-regulated condition as described above. The invention also provides an array of polypeptides, comprising at least two polypeptide species as recited above, wherein each polypeptide species is implicated in a hypoxia-regulated condition, or is a functional equivalent variant or fragment thereof.

Kits useful in the diagnostic methods of the invention may comprise such nucleic acid, antibody and/or polypeptide arrays.

According to the invention, a kit may also comprise one or more antibodies that bind to a polypeptide as recited above, and a reagent useful for the detection of a binding reaction between said antibody and said polypeptide.

According to a still further aspect of the invention, there is provided a genetically-modified non-human animal that has been transformed to express higher, lower or absent levels of a polypeptide according to any one of the aspects of the invention described above. Preferably, said genetically-modified animal is a transgenic or knockout animal.

The invention also provides a method for screening for a compound effective to treat a hypoxia-regulated condition, by contacting a non-human genetically-modified animal as described above with a candidate compound and determining the effect of the compound on the physiological state of the animal.

As discussed in some detail above, ischaemic disease pathologies involve a decrease in the blood supply to a bodily organ, tissue or body part generally caused by constriction or obstruction of the blood vessels. One particular example of an ischaemic disease pathology is myocardial ischaemia, which encompasses several chronic and acute cardiac pathologies that involve the deprivation of the myocardium of its blood supply, usually through coronary artery occlusion. A key component of ischaemia is hypoxia. Following transient ischaemia, the affected tissue may be subjected to reperfusion and re-oxygenation, and this is of significance in its own right.

Ischaemia/reperfusion is well known to induce cell death in myocardial tissue by apoptosis, leading to impaired function of the myocardium and infarction. Many of the specific molecules required to execute the process of apoptosis are known, but not all of these molecules have been characterised in detail. Cell death may also proceed by a distinct process called necrosis, which unlike apoptosis, is not initiated and controlled by specific and dedicated cellular and biochemical mechanisms (see Nicotera *et al.*, Biochem Soc Symp. 1999; 66:69-73). There is substantial evidence that apoptotic cell death occurs either during or after myocardial ischaemia (Kajstura *et al.*, Lab Invest. 1996; 74(1):86-107; Cheng *et al.*, Exp Cell Res. 1996; 226(2):316-27; Fliss and Gattinger, Circ Res. 1996; 79(5):949-56; Veinot *et al.*, Hum Pathol. 1997; 28(4):485-92; Bialik *et al.*, J Clin Invest. 1997; 100(6):1363-72; Gottlieb *et al.*, J Clin Invest. 1994; 94(4):1621-8; Gottlieb and Engler, Ann N Y Acad Sci. 1999; 874:412-26). In the laboratory, apoptosis is also induced by subjecting cardiac myocytes to hypoxia (Tanaka *et al.*, Circ Res. 1994 Sep;75(3):426-33; Long *et al.*, J Clin Invest. 1997 99(11): 2635-43).

Clearly, there is a significant clinical application were a successful method to inhibit apoptosis in ischaemic myocardial tissue to be devised. A specific and effective treatment requires identifying biochemical target(s), which are responsible for mediating apoptosis, specifically in ischaemic myocardial cells. One target which plays a common role in mediating apoptosis in many cell types, namely p53, is not involved in apoptosis resulting from myocardial ischaemia (Bialik *et al.*, J Clin Invest. 1997; 100(6):1363-72). Others have shown that inhibiting key mediators of apoptosis, caspases, provides protection against lethal reperfusion injury, following myocardial ischaemia in rat models (Mocanu *et al.*, Br J Pharmacol. 2000; 130(2):197-200; Yaoita *et al.*, Circulation. 1998 97(3): 276-81; Holly *et al.*, J Mol Cell Cardiol. 1999 31(9): 1709-15). However, this approach lacks specificity, since the caspases play a key role in mediating apoptosis in the majority of mammalian cell types, where it is usually beneficial. An

approach that involves modulating the activity of molecules shown specifically to mediate apoptosis in ischaemic cardiac cells, would present a distinct advantage in both specificity and efficacy.

It has now been discovered that a polypeptide encoded by a gene identified from the EST recited in SEQ ID No 86, having the Protein accession number BAB15101 (encoded by Homo sapiens cDNA: FLJ21620 fis, clone COL07838 Nucleotide accession AK025273) is regulated by hypoxia. Other public domain sequences corresponding to this gene include Homo sapiens cDNA: FLJ23265 fis, clone COL06456 Nucleotide accession AK026918. Accordingly, when referring in the present specification to the EST recited in SEQ ID No 86, it is intended that these gene and protein sequences are also embraced. This gene was identified using Research Genetics Human GeneFilters arrays, which contain an EST corresponding to the gene (accession number R00332). In the art, the gene is now termed EGL nine (C.elegans) homolog 3.

There are no reports that describe the function of this human gene. However, a high degree of amino acid homology is observed between the protein encoded by this gene, and a rat protein called "Growth factor responsive smooth muscle protein" or "SM20" (Nucleotide accession U06713; Protein accession A53770). An alignment of single letter amino acid sequences is shown below. Over the highlighted region there is 97% amino acid similarity and 96% amino acid identity.

```
A53770     (1) MTLRSRRGFLSFLPGLRPPRRWLRISKRGPPTSHWASPALGGRTLHYSCR
BAB15101   (1) ------------------------------------------------------
                51                                                100
A53770    (51) SQSGTPFSSEFQATFPAFAAKVARGPWLPQVVEPPARLSASPLCVRSGQA
BAB15101   (1) ------------------------------------------------------
                101                                               150
A53770    (101) LGACTLGVPRLGSVSEMPLGHIMRLDLEKIALEYIVPCLHEVGFCYLDNF
BAB15101   (1) ----------------MPLGHIMRLDLEKIALEYIVPCLHEVGFCYLDNF
                151                                               200
A53770    (151) LGEVVGDCVLERVKQLHYNGALRDGQLAGPRAGVSKRHLRGDQITWIGGN
BAB15101   (35) LGEVVGDCVLERVKQLHCTGALRDGQLAGPRAGVSKRHLRGDQITWIGGN
                201                                               250
A53770    (201) EEGCEAINFLLSLIDRLVLYCGSRLGKYYVKERSKAMVACYPGNGTGYVR
BAB15101   (85) EEGCEAISFLLSLIDRLVLYCGSRLGKYYVKERSKAMVACYPGNGTGYVR
                251                                               300
A53770    (251) HVDNPNGDGRCITCIYYLNKNWDAKLHGGVLRIFPEGKSFVADVEPIFDR
BAB15101  (135) HVDNPNGDGRCITCIYYLNKNWDAKLHGGILRIFPEGKSFIADVEPIFDR
                301                                               350
A53770    (301) LLFSWSDRRNPHEVQPSYATRYAMTVWYFDAEERAEAKKKFRNLTRKTES
BAB15101  (185) LLFFWSDRRNPHEVQPSYATRYAMTVWYFDAEERAEAKKKFRNLTRKTES
                351
A53770    (351) ALAKD
BAB15101  (235) ALTED
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The high degree of amino acid similarity suggests that the human protein BAB15101 has an equivalent biochemical function to the rat protein A53770 ("Growth factor responsive smooth muscle protein" or "SM20"). Recent publications have shown that SM20 functions to promote apoptosis in neurons (Lipscomb *et al.*, *J Neurochem* 1999; **73**(1):429-32; Lipscomb *et al.*, *J Biol Chem* 2000 Nov 1; [epub ahead of print]). Significantly, SM20 has been shown to be expressed at high levels in the heart (Wax *et al.*, *J Biol Chem* 1994; **269**(17): 13041-7).

It has also been discovered that a polypeptide encoded by a gene identified from the EST recited in SEQ ID No 90, having the Protein accession number CAB81622, is regulated by hypoxia. The encoding human gene has been annotated in the UniGene database as "Similar to rat smooth muscle protein SM-20"; the nucleotide sequence is contained within the nucleotide accession AL117352. More recently, a longer fragment of this gene has been cloned, named c1orf12, or EGLN1 (Nucleotide accession AAG34568; Protein accession AAG34568). Accordingly, when referring in the present specification to the EST recited in SEQ ID No 90, it is intended that these gene and protein sequences are also embraced.

This distinct human gene, encoding a protein related to SM20 and EGLN3 (BAB15101), is also induced in response to hypoxia. This gene was identified using Research Genetics Human GeneFilters arrays, which contain an EST corresponding to the gene (accession number H56028).

Independently to this, a fragment of this gene has been cloned from a cDNA library derived from hypoxic human cardiomyoblasts, and it has been shown that the gene is increased in expression in response to hypoxia in this cell type (see Table 1 herein; penultimate row). The nucleotide sequence of this cDNA fragment is referred to herein as SEQ ID No 90a.

In the light of this novel discovery reported herein that these human equivalents of SM20 are induced by hypoxia, it is herein proposed that in cardiac ischaemia, the resulting apoptosis is due at least in part, to increased expression of these genes.

The therapeutic modulation of the activity of EGLN3 (BAB15101), c1orf12 (AAG34568), CAB81622, SM20 and other equivalent proteins and encoding genes therefore provides a novel means for the treatment of myocardial ischaemia, through the alteration of the propensity of myocardial cells to undergo apoptosis. For example, a suitable treatment may involve altering the susceptibility of ischaemic myocardial tissue to subsequent reperfusion and re-oxygenation, or may involve modulating the susceptibility of chronic ischaemic myocardial tissue (including forms of angina) to later more severe ischaemia, which would result in myocardial infarction. It is submitted that, by way of analogy, cerebral ischaemia may be treated using the same principle.

These data provide the first connection between these related genes and the physiological response to hypoxia. Recently published research papers have identified that the protein products of these genes can

act as proline hydroxylases (see Bruick RK et al Science. 2001 294:1337-40 and Epstein AC et al Cell. 107:43-54). This is consistent with our observations that certain proline hydroxylases are induced in response to hypoxia and the genes EGLN1 and EGLN3 are part of the hypoxia response. For example, two genes encoding proline hydroxylases have been identified herein as being increased in expression in response to hypoxia (proline 4-hydroxylase, alpha polypeptide 1; SeqID: 231/232, proline 4-hydroxylase, alpha polypeptide II; SeqID: 349/ 350). This identified a functional significance of proline hydroxylation as a response to hypoxia. A preferred embodiment of the invention thus includes methods for modulating the biological response to hypoxia by modulating the proline hydroxylase activity of the EGLN3 (BAB15101), c1orf12 (AAG34568), CAB81622 and SM20 proteins.

Furthermore, a number of bacteria, such as *moraxella*, are thought to be involved in the initiation of inflammatory diseases. Many bacteria contain, within their genome, genes encoding proteins that share homology to the EGLN family of prolyl hydroxylases. We therefore propose that these bacterial genes may initiate a hypoxic like response at the site of infection thereby causing localised inflammation. The resulting inflammatory infiltrate could then cause the tissue to become hypoxic thereby continuing the cycle of hypoxia response.

As discussed in detail above, fragments and functional equivalents of the EGLN3 (BAB15101), c1orf12 (AAG34568), CAB81622, SM20 and other equivalent proteins are included within the present invention, in addition to ligands that bind specifically to these proteins. Furthermore, the invention also embraces purified and isolated nucleic acid molecules encoding these proteins, fragments and functional equivalents, vectors containing such nucleic acid molecules and host cells transformed with these vectors.

The therapeutic and diagnostic applications discussed above are also equally relevant to this aspect of the invention. For example, small molecule inhibitors of the EGLN3 (BAB15101), c1orf12 (AAG34568), CAB81622, SM20 and equivalent proteins and encoding genes are envisaged for utility as pharmaceutical agents, particularly in modulating the proline hydroxylase activity of the EGLN3 and c1orf12 proteins.

Truncated or chimeric inhibitory derivatives of the encoding genes, or distinct genes that encode regulators of the BAB15101, AAG34568, CAB81622 and SM20 encoding genes, are also envisaged for utility for gene therapy.

An alignment of the amino acid sequences of rat SM20 (Accession A53770), its human equivalent (Accession BAB15101; SEQ ID No: 85) and this distinct human homologue (Accession CAB81622 or AAG34568; SEQ ID No: 89) is shown below:

```
                1                                                  50
BAB15101    (1) --------------------------------------------------
  A53770    (1) --------------------------------------------------
AAG34568    (1) MANDSGGPGGPSPSERDRQYCELCGKMENLLRCSRCRSSFYCCKEHQRQD
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Consensus    (1)
                    51                                                  100
BAB15101     (1)  ----------------------------------------------------------
A53770       (1)  -----MTLRSRRGFLSFLPGLRPPRRWLRISKRGPPTSHWASP-----AL
AAG34568    (51)  WKKHKLVCQGSEGALGHGVGPHQHSGPAPPAAVPPPRAGAREPRKAAARR
Consensus   (51)       L      G L    G          A   PP A    P
                    101                                                 150
BAB15101     (1)  ----------------------------------------------------------
A53770      (41)  GGRTLHYSCRSQSGTPFSSEFQATFPAFAAKVARGPWLPQVVEPPAR---
AAG34568   (101)  DNASGDAAKGKVKAKPPADPAAAASPCRAAAGGQGSAVAAEAEPGKEEPP
Consensus  (101)     S   A      A P A    A  P  AA  A G  L    EP
                    151                                                 200
BAB15101     (1)  ----------------------------MPLGHIMRLDLEKIALEYIVP
A53770      (88)  LSASPLCVRSGQALGACTLGVPRLGSVSEMPLGHIMRLDLEKIALEYIVP
AAG34568   (151)  ARSSLFQEKANLYPPSNTPGDALSPGGGLRPNGQTKPLPALKLALEYIVP
Consensus  (151)    AS    KA     A T G          MPLGHIMRLDLEKIALEYIVP
                    201                                                 250
BAB15101    (22)  CLHEVGFCYLDNFLGEVVGDCVLERVKQLHCTGALRDGQLAGPRAGVSKR
A53770     (138)  CLHEVGFCYLDNFLGEVVGDCVLERVKQLHYNGALRDGQLAGPRAGVSKR
AAG34568   (201)  CMNKHGICVVDDFLGKETGQQIGDEVRALHDTGKFTDGQLVSQKS-DSSK
Consensus  (201)  CLHEVGFCYLDNFLGEVVGDCVLERVKQLH TGALRDGQLAGPRAGVSKR
                    251                                                 300
BAB15101    (72)  HLRGDQITWIGGNEEGCEAISFLLSLIDRLVLYCGSRLGKYYVKERSKAM
A53770     (188)  HLRGDQITWIGGNEEGCEAINFLLSLIDRLVLYCGSRLGKYYVKERSKAM
AAG34568   (250)  DIRGDKITWIEGKEPGCETIGLLMSSMDDLIRHCNGKLGSYKINGRTKAM
Consensus  (251)  HLRGDQITWIGGNEEGCEAI FLLSLIDRLVLYCGSRLGKYYVKERSKAM
                    301                                                 350
BAB15101   (122)  VACYPGNGTGYVRHVDNPNGDGRCITCIYYLNKNWDAKLHGGILRIFPEG
A53770     (238)  VACYPGNGTGYVRHVDNPNGDGRCITCIYYLNKNWDAKLHGGVLRIFPEG
AAG34568   (300)  VACYPGNGTGYVRHVDNPNGDGRCVTCIYYLNKDWDAKVSGGILRIFPEG
Consensus  (301)  VACYPGNGTGYVRHVDNPNGDGRCITCIYYLNKNWDAKLHGGILRIFPEG
                    351                                                 400
BAB15101   (172)  KSFIADVEPIFDRLLFFWSDRRNPHEVQPSYATRYAMTVWYFDAEERAEA
A53770     (288)  KSFVADVEPIFDRLLFSWSDRRNPHEVQPSYATRYAMTVWYFDAEERAEA
AAG34568   (350)  KAQFADIEPKFDRLLFFWSDRRNPHEVQPAYATRYAITVWYFDADERARA
Consensus  (351)  KSFIADVEPIFDRLLFFWSDRRNPHEVQPSYATRYAMTVWYFDAEERAEA
                    401                 427
BAB15101   (222)  KKKFRNLTRKTESALTED---------
A53770     (338)  KKKFRNLTRKTESALAKD---------
AAG34568   (400)  KVKYLTGEKGVRVELNKPSDSVGKDVF
Consensus  (401)  KKKFRNLTRKTESAL KD
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From this sequence alignment, a highly conserved region of amino acid sequence may be noted, the consensus of which is as follows:

KAMVACYPGNGTGYVRHVDNPNGDGRCITCIYYLNKNWDAKLHGGILRIFPEGKSFIADVEPIFDRLLFF WSDRRNPHEVQPSYATRYAMTVWYFDAEERAEAKKK

This consensus sequence, and variants thereof, may be used in the identification of other proteins that are implicated in the biological response to hypoxia. This aspect of the invention therefore provides a substantially purified polypeptide comprising the consensus sequence:

KAMVACYPGNGTGYVRHVDNPNGDGRCITCIYYLNKNWDAKLHGGILRIFPEGKSFIADVEPIFDRLLFF WSDRRNPHEVQPSYATRYAMTVWYFDAEERAEAKKK, or a variant thereof.

The invention also provides a substantially purified polypeptide comprising the consensus sequence: KAMVACYPGNGTGYVRHVDNPNGDGRCITCIYYLNKNWDAKLHGGILRIFPEGKSFIADVEPIFDRLLFF WSDRRNPHEVQPSYATRYAMTVWYFDAEERAEAKKK, or a variant thereof, in the treatment or diagnosis of a hypoxia-related disease or condition.

Neither this consensus domain nor any proteins that contain this domain have been previously associated with the cellular response to hypoxia/ischaemia. Searches of the public databases indicate that the human genome contains several genes that encode proteins that contain this consensus sequence. These proteins may have similar functions or may function in the same biochemical pathway, potentially with an antagonistic effect.

By "variant" is meant a variation of the consensus sequence given above, that exhibits a degree of homology with the consensus sequence above a certain threshold level of identity or similarity. Degrees of identity and similarity can be readily calculated according to methods known in the art (see, for example, Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing. Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York, 1993). Typically, greater than 50% identity between two sequences is considered to be an indication of functional equivalence. Preferably, a variant consensus according to this aspect of the invention exhibits a degree of sequence identity with the consensus sequence given above, of greater than 50%. More preferred polypeptides have degrees of identity of greater than 60%, 70%, 80%, 90%, 95%, 98% or 99%, respectively.

As discussed in detail above, fragments and functional equivalents of these proteins are included within the present invention, in addition to ligands that bind specifically to these proteins. Furthermore, the invention also embraces purified and isolated nucleic acid molecules encoding these proteins, fragments and functional equivalents, vectors containing such nucleic acid molecules and host cells transformed with these vectors. The therapeutic and diagnostic applications discussed above are also equally relevant to this aspect of the invention.

The polypeptide referred to above as that encoded by SEQ ID No 91 is a specific protein that is termed "Semaphorin 4b". The gene encoding this protein is regulated (activated) by conditions of hypoxia. The Semaphorin 4b protein is encoded by a gene identified from the EST recited in SEQ ID No 92. The unequivocal and accurate full length cDNA sequence is provided herein as SEQ ID No 92a. The accurate presumptive amino acid sequence is provided herein as SEQ ID No 91. This protein, functionally-equivalent variants of this protein, the encoding nucleic acid molecules and ligands that regulate the activity and/or expression of this gene and protein are claimed above in the context of their role in hypoxia and hypoxia-related disorders.

Semaphorins are a large family of proteins, characterised by the 500 amino acid sema domain (Puschel et al., 1995, Neuron, 14(5): 941-8; Tamagnone and Comoglio, 2000, Trends Cell Biol., 10(9): 377-83). Early work showed a role in the guidance of axons during brain development, and the regulation of cell migration. More recently, specific members of this large family have been associated with cancer (Brambilla et al., Am J Pathol., 2000, 156(3): 939-50), rheumatoid arthritis (Mangasser-Stephan et al., Biochem Biophys Res Commun., 1997, 234(1): 153-6), the immune system (Spriggs, Curr Opin Immunol., 1999, 11(4): 387-91) including B-lymphocyte functions (Hall et al., Proc Natl Acad Sci U S A, 1996, 93(21): 11780-5) and angiogenesis (Miao et al., J Cell Biol., 1999, 146(1): 233-42). This is perhaps not surprising considering that cell migration / trafficking is a key part of inflammation, angiogenesis and tumour metastasis.

There are at least distinct 25 human semaphorin genes and the significance/ utility of many of these remains untested. This includes the Semaphorin 4b protein, which is unpublished and until now has not been assigned a full and accurate amino acid sequence.

We have made experimental discoveries which link the expression of Semaphorin 4b to factors (hypoxia, gamma IFN and superoxide radicals) that are associated with a variety of human ischaemic and inflammatory diseases. In particular, a key response of cells to hypoxia is to stimulate angiogenesis, and a key part of inflammation is the recruitment and trafficking of immune cells. In light of our discoveries, and what is known about other specific members of the semaphorin family, it is herein proposed that Semaphorin 4b is a regulator of these cellular functions, and thus provides a novel target for therapeutic intervention. This paves the way for the development of therapeutic agents that either potentiate or antagonise functions of Semaphorin 4b. Such agents are likely to be highly valuable in the treatment of human disease.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA technology and immunology, which are within the skill of those working in the art.

Most general molecular biology, microbiology recombinant DNA technology and immunological techniques can be found in Sambrook *et al.*, Molecular Cloning, A Laboratory Manual (1989) Cold Harbor-Laboratory Press, Cold Spring Harbor, N.Y. or Ausubel *et al.*, Current protocols in molecular biology (1990) John Wiley and Sons, N.Y.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

A. Polypeptides

The term "polypeptide" as used herein, refers to a chain (may be branched or unbranched) of two or more amino acids linked to each other by means of a peptide bond or modified peptide bond (isosteres). The term polypeptide encompasses but is not limited to oligopeptides, peptides and proteins. The polypeptide of the invention may additionally be either in a mature protein form or in a pre-, pro- or prepro-protein form that requires subsequent cleavage for formation of the active mature protein. The pre-, pro-, prepro-part of the protein is often a leader or secretory sequence but may also be an additional sequence added to aid protein purification (for example, a His tag) or to conform a higher stability to the protein.

A polypeptide according to the invention may also include modified amino acids, that is, amino acids other than those 20 that are gene-encoded. This modification may be a result of natural processes such as post-translational processing or by chemical modification. Examples of modifications include acetylation, acylation, amidation, ADP-ribosylation, arginylation, attachment of a lipid derivative or phosphatidylinositol, γ-carboxylation, covalent attachment of a flavin or haeme moiety, a nucleotide or nucleotide derivative, cyclisation, demethylation, disulphide bond formation, formation of covalent cross-links, formylation, glycosylation, GPI anchor formation, hydroxylation, iodination, lipid attachment, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemisation, selenoylation, sulphation, and ubiquitination. Modification of the polypeptide can occur anywhere within the molecule including the backbone, the amino acid side-chains or at the N- or C-terminals.

A polypeptide according to the invention may either be isolated from natural sources (for example, purified from cell culture), or be a recombinantly produced polypeptide, or a synthetically produced polypeptide or a combination of all the above.

Antibodies

A polypeptide according to the invention, its functional equivalents and/or any immunogenic fragments derived from the polypeptide may be used to generate ligands including immunospecific monoclonal or polyclonal antibodies, or antibody fragments. These antibodies can then be used to isolate or identify

clones expressing the polypeptide of the invention or to purify the polypeptide by affinity chromatography. Further uses of these immunospecific antibodies may include, but are not limited to, diagnostic, therapeutic or general assay applications. Examples of assay techniques that employ antibodies are immunoassays, radioimmunoassays (RIA) or enzyme linked immunosorbent assay (ELISA). In these cases, the antibodies may be labelled with an analytically-detectable reagent including radioisotopes, a fluorescent molecule or any reporter molecule.

The term "immunospecific" as used herein refers to antibodies that have a substantially higher affinity for a polypeptide of this invention compared with other polypeptides. The term "antibody" as used herein refers to a molecule that is produced by animals in response to an antigen and has the particular property of interacting specifically with the antigenic determinant that induced its formation. Fragments of the aforementioned molecule such as Fab, $F(ab')_2$ and scFv, which are capable of binding the antigen determinant, are also included in the term "antibody". Antibodies may also be modified to make chimeric antibodies, where non-human variable regions are joined or fused to human constant regions (for example, Liu *et al.*, PNAS, USA, 84, 3439 (1987)). Particularly, antibodies may be modified to make them less immunogenic to an individual in a process such as humanisation (see, for example, Jones *et al.*, Nature, 321, 522 (1986); Verhoeyen *et al.*, Science, 239, 1534 (1988); Kabat *et al.*, J. Immunol., 147, 1709 (1991); Queen *et al.*, PNAS, USA, 86, 10029 (1989); Gorman *et al.*, PNAS, USA, 88, 34181 (1991) and Hodgson *et al.*, Bio/Technology, 9, 421 (1991)). The term "humanised antibody", as used herein, refers to antibody molecules in which the amino acids of the CDR (complementarity-determining region) and selected other regions in the variable domains of the heavy and/or light chains of a non-human donor antibody have been substituted with the equivalent amino acids of a human antibody. The humanised antibody therefore closely resembles a human antibody, but has the binding ability of the donor antibody. Antibodies may also have a "bispecific" nature, that is, the antibody has two different antigen binding domains, each domain being directed against a different epitope.

Specific polyclonal antibodies may be made by immuno-challenging an animal with a polypeptide of this invention. Common animals used for the production of antibodies include the mouse, rat, chicken, rabbit, goat and horse. The polypeptide used to immuno-challenge the animal may be derived by recombinant DNA technology or may be chemically-synthesised. In addition, the polypeptide may be conjugated to a carrier protein. Commonly used carriers to which the polypeptides may be conjugated include, but are not limited to BSA (bovine serum albumin), thyroglobulin and keyhole limpet haemocyanin. Serum from the immuno-challenged animal is collected and treated according to known procedures, for example, by immunoaffinity chromatography.

Specific monoclonal antibodies can generally be made by methods known to one skilled in the art (see for

example, Kohler, G. and Milstein, C., Nature 256, 495-497 (1975); Kozbor *et al.*, Immunology Today 4: 72 (1983); Cole *et al.*, 77-96 in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc. (1985) and Roitt, I. *et al.*, Immunology, 25.10, Mosby-Year Book Europe Limited (1993)). Panels of monoclonal antibodies produced against the polypeptides of the invention can be screened for various properties, i.e., for isotype, epitope, affinity, etc. against which they are directed. Alternatively, genes encoding the monoclonal antibodies of interest may be isolated from hybridomas, for instance using PCR techniques known in the art, and cloned and expressed in appropriate vectors.

Phage display technology may be utilised to select the genes encoding the antibodies that have exhibited an immunspecific response to the polypeptides of the invention (see McCafferty, J., *et al.*, (1990), Nature 348, 552-554; Marks, J. *et al.*, (1992) Biotechnology 10, 779-783).

Ligands

The polypeptides of the invention may also be used to search for interacting ligands. Methods for doing this include the screening of a library of compounds (see Coligan *et al.*, Current Protocols in Immunology 1(2); Chapter 5 (1991), isolating the ligands from cells, isolating the ligands from a cell-free preparation or natural product mixtures. Ligands to the polypeptide may activate (agonise) or inhibit (antagonise) its activity. Alternatively, compounds may affect the levels of the polypeptide present in the cell, including affecting gene expression, mRNA stability and the degree of post-translational modification of the encoded protein. The invention thus embraces methods for the identification of a compound that is effective in the treatment and/or diagnosis of disease, comprising contacting a polypeptide, a nucleic acid molecule or host cell according to any one of the embodiments of the invention described herein with one or more compounds suspected of possessing binding affinity for said polypeptide or nucleic acid molecule, and selecting a compound that binds specifically to said nucleic acid molecule or polypeptide, or that affects the level of gene expression, mRNA stability or the degree of post-translational modification of the encoded protein.

Ligands to the polypeptide form a further aspect of the invention, as discussed in more detail above. Preferred "antagonist" ligands include those that bind to the polypeptide of this invention and strongly inhibit any activity of the polypeptide. Preferred "agonist" ligands include those that bind to the polypeptide and strongly induce activity of the polypeptide of this invention or increases substantially the level of the polypeptide in the cell. As defined above, the term "agonist" is meant to include any polypeptide, peptide, synthetic molecule or organic molecule that functions as an activator, by increasing the effective biological activity of a polypeptide, for example, by increasing gene expression or enzymatic activity. The term "antagonist" is meant to include any polypeptide, peptide, synthetic molecule or

organic molecule that functions as an inhibitor, by decreasing the effective biological activity of the gene product, for example, by inhibiting gene expression of an enzyme or a pharmacological receptor.

Ligands to a polypeptide according to the invention may come in various forms, including natural or modified substrates, enzymes, receptors, small organic molecules such as small natural or synthetic organic molecules of up to 2000Da, preferably 800Da or less, peptidomimetics, inorganic molecules, peptides, polypeptides, antibodies, structural or functional mimetics of the aforementioned.

B. Nucleic acid molecules

Preferred nucleic acid molecules of the invention are those which encode the polypeptide sequences recited in any one of SEQ ID Nos. 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 63, 67, 69, 73, 75, 77, 85, 87, 89, 91, 93, 95, 99, 103, 113, 115, 119, 121, 129, 131, 133, 137, 139, 141, 145, 151, 153, 157, 159, 163, 169, 181, 187, 201, 205, 207 and 209. Examples of such nucleic acid molecules include those listed in SEQ ID Nos. 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 92a, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214 and 216, homologous nucleic acids and nucleic acids that are complementary to these nucleic acid molecules. Nucleic acid molecules of this aspect of the invention may be used in numerous methods and applications, as described generally herein. A nucleic acid molecule preferably comprises of at least *n* consecutive nucleotides from any one of the sequences disclosed in SEQ ID Nos. 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 92a, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214 and 216, where *n* is 10 or more. A nucleic acid molecule of the invention also includes sequences that are complementary to the nucleic acid molecule described above (for example, for antisense or probing purposes).

A nucleic acid molecule according to this aspect of the invention may be in the form of RNA, such as mRNA, DNA, such as cDNA, synthetic DNA or genomic DNA. The nucleic acid molecule may be double-stranded or single-stranded. The single-stranded form may be the coding (sense) strand or the non-coding (antisense) strand. A nucleic acid molecule may also comprise an analogue of DNA or RNA, including, but not limited to modifications made to the backbone of the molecule, such as, for example, a peptide nucleic acid (PNA). The term "PNA" as used herein, refers to an antisense molecule that

comprises an oligonucleotide of at least five nucleotides in length linked to a peptide backbone of amino acid residues, preferably ending in lysine. The terminal lysine confers solubility to the composition. PNAs may be pegylated to extend their lifespan in a cell, where they preferentially bind complementary single-stranded DNA and RNA and stop transcript elongation (Nielsen, P.E. *et al.* (1993) Anticancer Drug Des. 8:53-63).

A nucleic acid molecule according to this aspect of the invention can be isolated by cloning, purification or separation of the molecule directly from a particular organism, or from a library, such as a genomic or cDNA library. The molecule may also be synthesised, for example, using chemical synthetic techniques such as solid phase phosphoramidite chemical synthesis. RNA may be synthesized *in vitro* or *in vivo* by transcription of the relevant DNA molecule.

Due to the degeneracy of the genetic code, differing nucleic acid sequences may encode the same polypeptide (or mature polypeptide). Thus, nucleic acid molecules included in this aspect of the invention include any molecule comprising a variant of the sequence explicitly recited. Such variants may include variant nucleic acid molecules that code for the same polypeptide (or mature polypeptide) as that explicitly identified, that code for a fragment of the polypeptide, that code for a functional equivalent of the polypeptide or that code for a fragment of the functional equivalent of the polypeptide. Also included in this aspect of the invention, are variant nucleic acid molecules that are derived from nucleotide substitutions, deletions, rearrangements or insertions or multiple combinations of the aforementioned. Such molecules may be naturally occurring variants, such as allelic variants, non-naturally occurring variants such as those created by chemical mutagenesis, or variants isolated from a species, cell or organism type other than the type from which the sequence explicitly identified originated. Variant nucleic acid molecules may differ from the nucleic acid molecule explicitly recited in a coding region, non-coding region or both these regions.

Nucleic acid molecules may also include additional nucleic acid sequence to that explicitly recited, for example, at the 5' or 3' end of the molecule. Such additional nucleic acids may encode for a polypeptide with added functionality compared with the original polypeptide whose sequence is explicitly identified herein. An example of this would be an addition of a sequence that is heterologous to the original nucleic acid sequence, to encode a fusion protein. Such a fusion protein may be of use in aiding purification procedures or enabling techniques to be carried out where fusion proteins are required (such as in the yeast two hybrid system). Additional sequences may also include leader or secretory sequences such as those coding for pro-, pre- or prepro- polypeptide sequences. These additional sequences may also include non-coding sequences that are transcribed but not translated including ribosome binding sites and termination signals.

A nucleic acid molecule of the invention may include molecules that are at least 70% identical over their entire length to a nucleic acid molecule as explicitly identified herein in SEQ ID Nos.: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 92a, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214 and 216. Preferably, a nucleic acid molecule according to this aspect of the invention comprises a region that is at least 80% identical over its entire length to a nucleic acid molecule as explicitly identified herein in these SEQ ID Nos., preferably at least 90%, more preferably at least 95% and most preferably at least 98% or 99% identical. Further preferred embodiments include nucleic acid molecules that encode polypeptides that retain substantially the same biological function or activity as the polypeptide explicitly identified herein. The terms "homology" and "identity" should be given the meanings described in detail above with respect to polypeptide analysis. Preferably, nucleotide homology and identity are assessed using the blastn program available at http://www.ncbi.nlm.nih.gov.

The nucleic acid molecules of the invention can also be engineered using methods generally known in the art. These methods include but are not limited to DNA shuffling; random or non-random fragmentation (by restriction enzymes or shearing methods) and reassembly of fragments; insertions, deletions, substitutions and rearrangements of sequences by site-directed mutagenesis (for example, by PCR). These alterations may be for a number of reasons including for ease of cloning (such as introduction of new restriction sites), altering of glycosylation patterns, changing of codon preferences, splice variants changing the processing, and/or expression of the gene product (the polypeptide) in general or creating fusion proteins (see above).

Hybridisation

Nucleic acid molecules of the invention may also include antisense molecules that are partially complementary to a nucleic acid molecule as explicitly identified herein in SEQ ID Nos.: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 92a, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214 and 216, and which therefore will hybridise to the encoding nucleic acid molecules. These antisense molecules, including oligonucleotides, can be designed to recognise, specifically bind to and prevent transcription of a target nucleic acid encoding a polypeptide of the invention, as will be known by those of ordinary skill in the art (see Cohen, J.S., Trends in Pharm. Sci., 10, 435 (1989), Okano, J. Neurochem. 56, 560 (1991); O'Connor, J. Neurochem 56, 560 (1991); Lee

et al., Nucleic Acids Res 6, 3073 (1979); Cooney *et al.*, Science 241, 456 (1988); Dervan *et al.*, Science 251, 1360 (1991).

The term "hybridisation" used herein refers to any process by which a strand of nucleic acid binds with a complementary strand of nucleic acid by hydrogen bonding, typically forming Watson-Crick base pairs. As carried out *in vitro*, one of the nucleic acid populations is usually immobilised to a surface, whilst the other population is free. The two molecule types are then placed together under conditions conducive to binding.

The phrase "stringency of hybridisation" refers to the percentage of complementarity that is needed for duplex formation. "Stringency" thus refers to the conditions in a hybridization reaction that favour the association of very similar molecules over association of molecules that differ. Conditions can therefore exist that allow not only nucleic acid strands with 99-100% complementarity to hybridise, but sequences with lower complementarity (for example, 50%) to also hybridise. High stringency hybridisation conditions are defined herein as overnight incubation at 42°C in a solution comprising 50% formamide, 5XSSC (150mM NaCl, 15mM trisodium citrate), 50mM sodium phosphate (pH7.6), 5x Denhardts solution, 10% dextran sulphate, and 20 microgram/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1X SSC at approximately 65°C. Low stringency conditions involve the hybridisation reaction being carried out at 35°C (see Sambrook *et al.* [supra]). Preferably, the conditions used for hybridization are those of high stringency.

Some *trans*- and *cis*-acting factors that may affect the binding of two complementary strands include strand length, base composition (GC pairs have an extra hydrogen bond and are thus require more energy to separate than AT pairs) and the chemical environment. The presence of monovalent cations (such as Na^+) stabilises duplex formation whereas chemical denaturants such as formamide and urea destabilise the duplex by disruption of the hydrogen bonds. Use of compounds such as polyethylene glycol (PEG) can increase reassociation speeds by increasing overall DNA concentration in aqueous solution by abstracting water molecules. Denhardt's reagent or BLOTTO are chemical agents often added to block non-specific attachment of the liquid phase to the solid support. Increasing the temperature will also increase the stringency of hybridisation, as will increasing the stringency of the washing conditions following hybridisation (Sambrook *et al.* [*supra*]).

Numerous techniques exist for effecting hybridisation of nucleic acid molecules. Such techniques usually involve one of the nucleic acid populations being labelled. Labelling methods include, but are not limited to radiolabelling, fluorescence labelling, chemiluminescent or chromogenic labelling or chemically coupling a modified reporter molecule to a nucleotide precursor such as the biotin-streptavidin system.

This can be done by oligolabelling, nick-translation, end-labelling or PCR amplification using a labelled polynucleotide. Labelling of RNA molecules can be achieved by cloning the sequences encoding the polypeptide of the invention into a vector specifically for this purpose. Such vectors are known in the art and may be used to synthesise RNA probes *in vitro* by the addition of an appropriate RNA polymerase such as T7, T3 or SP6 and labelled nucleotides.

Various kits are commercially available that allow the labelling of molecules. Examples include those made by Pharmacia & Upjohn (Kalamazoo, MI); Promega (Madison WI); and the U.S. Biochemical Corp. (Cleveland, OH). Hybridisation assays include, but are not limited to dot-blots, Southern blotting, Northern blotting, chromosome *in situ* hybridisation (for example, FISH [fluorescence *in situ* hybridisation]), tissue *in situ* hybridisation, colony blots, plaque lifts, gridded clone hybridisation assays, DNA microarrays and oligonucleotide microarrays. These hybridisation methods and others, may be used by a skilled artisan to isolate copies of genomic DNA, cDNA, or RNA encoding homologous or orthologous proteins from other species.

The invention therefore also embodies a process for detecting a nucleic acid molecule according to the invention, comprising the steps of: (a) contacting a nucleic probe with a biological sample under hybridising conditions to form duplexes: and (b) detecting any such duplexes that are formed. The term "probe" as used herein refers to a nucleic acid molecule in a hybridisation reaction whose molecular identity is known and is designed specifically to identify nucleic acids encoding homologous genes in other species. Usually, the probe population is the labelled population, but this is not always the case, as for example, in a reverse hybridisation assay.

One example of a use of a probe is to find nucleic acid molecules with an equivalent function to those that are explicitly identified herein, or to identify additional family members in the same or other species. This can be done by probing libraries, such as genomic or cDNA libraries, derived from a source of interest, such as a human, a non-human animal, other eukaryote species, a plant, a prokaryotic species or a virus. The probe may be natural or artificially designed using methods recognised in the art (for example, Ausubel *et al.*, [*supra*]). A nucleic acid probe will preferably possess greater than 15, more preferably greater than 30 and most preferably greater than 50 contiguous bases complementary to a nucleic acid molecule explicitly identified herein.

In many cases, isolated DNA from cDNA libraries will be incomplete in the region encoding the polypeptide, normally at the 5' end. Methods available for subsequently obtaining full-length cDNA sequence include RACE (rapid amplification of cDNA ends) as described by Frohman *et al.*, (Proc. Natl. Acad. Sci. USA 85, 8998-9002 (1988)), and restriction-site PCR, which uses universal primers to retrieve

unknown nucleic acid sequence adjacent to a known locus (Sarkar, G. (1993) PCR Methods Applic., 2:318-322). "Inverse PCR" may also be used to amplify or to extend sequences using divergent primers based on a known region (Triglia, T. *et al.*, (1988) Nucleic Acids Res. 16:8186). Another method which may be used is "capture PCR", which involves PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA (Lagerstrom, M. *et al.*, (1991) PCR Methods Applic., 1:111-119). Another method which may be used to retrieve unknown sequences is that of Parker, J.D. *et al.*, (1991); Nucleic Acids Res. 19:3055-3060). Additionally, one may use PCR, nested primers, and libraries, such as the PromoterFinder™ library (Clontech, Palo Alto, CA) to walk genomic DNA. This latter process avoids the need to screen libraries and is useful in finding intron/exon junctions.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. Also, random-primed libraries are preferable, in that they will contain more sequences that contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into 5' non-transcribed regulatory regions.

In one embodiment of the invention, a nucleic acid molecule according to the invention may be used for chromosome localisation. In this technique, a nucleic acid molecule is specifically targeted to, and can hybridise with, a particular location on an individual human chromosome. The mapping of relevant sequences to chromosomes is an important step in the confirmatory correlation of those sequences with the gene-associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found in, for example, McKusick, Mendelian Inheritance in Man (available on-line through Johns Hopkins University Welch Medical Library). The relationships between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes). This provides valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the disease or syndrome has been crudely localised by genetic linkage to a particular genomic region, any sequences mapping to that area may represent associated or regulatory genes for further investigation. The nucleic acid molecule may also be used to detect differences in the chromosomal location due to translocation, inversion, etc. among normal, carrier, or affected individuals.

Nucleic acid molecules of the present invention are also valuable for tissue localisation. Such techniques facilitate the determination of expression patterns of the polypeptide in tissues by detection of the mRNAs that encode them. These techniques include *in situ* hybridisation techniques and nucleotide amplification techniques, such as PCR. Results from these studies provide an indication of the normal functions of the

polypeptide in the organism, as well as highlighting the involvement of a particular gene in a disease state or abnormal physiological condition.

In addition, comparative studies of the normal expression pattern of mRNAs with that of mRNAs encoded by a mutant gene provide valuable insights into the role of mutant polypeptides in disease. Such inappropriate expression may be of a temporal, spatial or quantitative nature.

Vectors

The nucleic acid molecules of the present invention may be incorporated into vectors for cloning (for example, pBluescript made by Stratagene) or expression purposes. Vectors containing a nucleic acid molecule explicitly identified herein (or a variant thereof) form another aspect of this invention. The nucleic acid molecule may be inserted into an appropriate vector by any variety of well known techniques such as those described in Sambrook *et al.* [supra]. Generally, the encoding gene can be placed under the control of a control element such as a promoter, ribosome binding site or operator, so that the DNA sequence encoding the desired polypeptide is transcribed into RNA in the transformed host cell.

Vectors may be derived from various sources including, but not limited to bacterial plasmids, bacteriophage, transposons, yeast episomes, insertion elements, yeast chromosomal elements, viruses for example, baculoviruses and SV40 (simian virus), vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses, lentiviruses and retroviruses, or combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, including cosmids and phagemids. Human, bacterial and yeast artificial chromosomes (HACs, BACs and YACs respectively) may also be employed to deliver larger fragments of DNA than can be contained and expressed in a plasmid.

Examples of retroviruses include but are not limited to: murine leukaemia virus (MLV), human immunodeficiency virus (HIV), equine infectious anaemia virus (EIAV), mouse mammary tumour virus (MMTV), Rous sarcoma virus (RSV), Fujinami sarcoma virus (FuSV), Moloney murine leukaemia virus (Mo-MLV), FBR murine osteosarcoma virus (FBR MSV), Moloney murine sarcoma virus (Mo-MSV), Abelson murine leukaemia virus (A-MLV), Avian myelocytomatosis virus-29 (MC29), and Avian erythroblastosis virus (AEV). A detailed list of retroviruses may be found in Coffin et al ("Retroviruses" 1997 Cold Spring Harbour Laboratory Press Eds: JM Coffin, SM Hughes, HE Varmus pp 758-763).

Lentiviruses can be divided into primate and non-primate groups. Examples of primate lentiviruses include but are not limited to: the human immunodeficiency virus (HIV), the causative agent of human auto-immunodeficiency syndrome (AIDS), and the simian immunodeficiency virus (SIV). The non-primate lentiviral group includes the prototype "slow virus" visna/maedi virus (VMV), as well as the related caprine arthritis-encephalitis virus (CAEV), equine infectious anaemia virus (EIAV) and the more recently described feline immunodeficiency virus (FIV) and bovine immunodeficiency virus (BIV).

A distinction between the lentivirus family and other types of retroviruses is that lentiviruses have the capability to infect both dividing and non-dividing cells (Lewis et al 1992 EMBO. J 11: 3053-3058; Lewis and Emerman 1994 J. Virol. 68: 510-516). In contrast, other retroviruses - such as MLV - are unable to infect non-dividing cells such as those that make up, for example, muscle, brain, lung and liver tissue.

A vector may be configured as a split-intron vector. A split intron vector is described in PCT patent applications WO 99/15683 and WO 99/15684.

If the features of adenoviruses are combined with the genetic stability of retroviruses/lentiviruses then essentially the adenovirus can be used to transduce target cells to become transient retroviral producer cells that could stably infect neighbouring cells. Such retroviral producer cells engineered to express an antigen of the present invention can be implanted in organisms such as animals or humans for use in the treatment of angiogenesis and/or cancer.

Poxvirus vectors are also suitable for use in accordance with the present invention. Pox viruses are engineered for recombinant gene expression and for the use as recombinant live vaccines. This entails the use of recombinant techniques to introduce nucleic acids encoding foreign antigens into the genome of the pox virus. If the nucleic acid is integrated at a site in the viral DNA which is non-essential for the life cycle of the virus, it is possible for the newly produced recombinant pox virus to be infectious, that is to say to infect foreign cells and thus to express the integrated DNA sequence. The recombinant pox virus prepared in this way can be used as live vaccines for the prophylaxis and/or treatment of pathologic and infectious disease.

For vaccine delivery, preferred vectors are vaccinia virus vectors such as MVA or NYVAC. Most preferred is the vaccinia strain modified virus ankara (MVA) or a strain derived therefrom. Alternatives to vaccinia vectors include avipox vectors such as fowlpox or canarypox known as ALVAC and strains derived therefrom which can infect and express recombinant proteins in human cells but are unable to replicate.

Bacterial vectors may be also used, such as salmonella, listeria and mycobacteria.

Vectors containing the relevant nucleotide sequence may enter the host cell by a variety of methods well known in the art and described in many standard laboratory manuals (such as Sambrook *et al.*, [supra], Ausubel *et al.*, [supra], Davis *et al.*, Basic Methods in Molecular Biology (1986)). Methods include calcium phosphate transfection, cationic lipid-mediated transfection, DEAE-dextran mediated transfection, electroporation, microinjection, scrape loading, transduction, and ballistic introduction or

infection.

Host cells

The choice of host cells is often dependent on the vector type used as a carrier for the nucleic acid molecule of the present invention. Bacteria and other microorganisms are particularly suitable hosts for plasmids, cosmids and expression vectors generally (for example, vectors derived from the pBR322 plasmid), yeast are suitable hosts for yeast expression vectors, insect cell systems are suitable host for virus expression vectors (for example, baculovirus) and plant cells are suitable hosts for vectors such as the cauliflower mosaic virus (CaMV) and tobacco mosaic virus (TMV). Other expression systems include using animal cells (for example, with the LentiVectors™, Oxford BioMedica) as a host cell or even using cell-free translating systems. Some vectors, such as "shuttle vectors" may be maintained in a variety of host cells. An example of such a vector would be pEG 202 and other yeast two-hybrid vectors which can be maintained in both yeast and bacterial cells (see Ausubel *et al.*, [supra] and Gyuris, J., Cell, 75, 791-803).

Examples of suitable bacterial hosts include *Streptococci, Staphylococci, Escherichia coli, Streptomyces* and *Bacillus subtilis* cells. Yeast and fungal hosts *include Saccharomyces cerevisiae* and *Aspergillus* cells. Mammalian cell hosts include many immortalised cell lines available from the American Type Culture Collection (ATCC) such as CHO (Chinese Hamster Ovary) cells, HeLa cells, BHK (baby hamster kidney) cells, monkey kidney cells, C127, 3T3, BHK, HEK 293, Bowes melanoma and human hepatocellular carcinoma (for example, Hep G2) cells. Insect host cells that are used for baculovirus expression include Drosophila S2 and Spodoptera Sf9 cells. Plant host cells include most plants from which protoplasts be isolated and cultured to give whole regenerated plants. Practically, all plants can be regenerated from cultured cells or tissues, including but not limited to all major species of sugar cane, sugar beet, cotton, fruit and other trees, legumes and vegetables.

Expression systems

Also included in present invention are expression vectors that comprise a nucleic acid molecule as described above. Expression vectors and host cells are preferably chosen to give long term, high yield production and stable expression of the recombinant polypeptide and its variants.

Expression of a polypeptide can be effected by cloning an encoding nucleic acid molecule into a suitable expression vector and inserting this vector into a suitable host cell. The positioning and orientation of the nucleic acid molecule insert with respect to the regulatory sequences of the vector is important to ensure that the coding sequence is properly transcribed and translated. Alternatively, control and other regulatory

sequences may be ligated onto the nucleic acid molecule of this invention prior to its insertion into the expression vector. In both cases, the sequence of the nucleic acid molecule may have to be adjusted in order to effect correct transcription and translation (for example, addition of nucleotides may be necessary to obtain the correct reading frame for translation of the polypeptide from its encoding nucleic acid molecule).

A nucleic acid molecule of the invention may comprise control sequences that encode signal peptides or leader sequences. These sequences may be useful in directing the translated polypeptide to a variety of locations within or outside the host cell, such as to the lumen of the endoplasmic reticulum, to the nucleus, to the periplasmic space, or into the extracellular environment. Such signals may be endogenous to the nucleic acid molecules of the invention, or may be a heterologous sequence. These leader or control sequences may be removed by the host during post-translational processing.

A nucleic acid molecule of the present invention may also comprise one or more regulatory sequences that allow for regulation of the expression of polypeptide relative to the growth of the host cell. Alternatively, these regulatory signals may be due to a heterologous sequence from the vector. Stimuli that these sequences respond to include those of a physical or chemical nature such as the presence or absence of regulatory compounds, changing temperatures or metabolic conditions. Regulatory sequences as described herein, are non-translated regions of sequence such as enhancers, promoters and the 5' and 3' untranslated regions of genes. Regulatory sequences interact with host cellular proteins that carry out translation and transcription. These regulatory sequences may vary in strength and specificity. Examples of regulatory sequences include those of constitutive and inducible promoters. In bacterial systems, an example of an inducible promoter is the hybrid *lacZ* promoter of the Bluescript phagemid (Stratagene, LaJolla, CA) or pSportlTM plasmid (Gibco BRL). The baculovirus polyhedrin promoter may be used in insect cells.

An example of a preferred expression system is the lentivirus expression system, for example, as described in International patent application WO98/17815.

Detection of uptake of vectors by the host organism

Various methods are known in the art to detect the uptake of a nucleic acid or vector molecule by a host cell and/or the subsequent successful expression of the encoded polypeptide (see for example Sambrook *et al.*, [supra]).

Vectors frequently have marker genes that can be easily assayed. Thus, vector uptake by a host cell can be readily detected by testing for the relevant phenotype. Markers include, but are not limited to those coding for antibiotic resistance, herbicide resistance or nutritional requirements. The gene encoding

dihydrofolate reductase (DHFR) for example, confers resistance to methotrexate (Wigler, M. *et al.* (1980) PNAS 77:3567-70) and the gene *npt* confers resistance to the aminoglycosides neomycin and G-418 (Colbere-Garapin, F. *et al* (1981) J. Mol. Biol. 150:1-14). Additional selectable genes have been described, examples of which will be clear to those of skill in the art.

Markers however, only indicate that a vector has been taken up by a host cell but does not distinguish between vectors that contain the desired nucleic acid molecule and those that do not. One method of detecting for the said nucleic acid molecule is to insert the relevant sequence at a position that will disrupt the transcription and translation of a marker gene. These cells can then be identified by the absence of a marker gene phenotype. Alternatively, a marker gene can be placed in tandem with a sequence encoding a polypeptide of the invention under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

More direct and definitive methods to detect the presence of the nucleic acid molecule of the present invention include DNA-DNA or DNA-RNA hybridisation with a probe comprising the relevant antisense molecule, as described above. More direct methods to detect polypeptide expression include protein bioassays for example, fluorescence activated cell sorting (FACS), immunoassay techniques such as ELISA or radioimmunoassays.

Alternative methods for detecting or quantitating the presence of the nucleic acid molecule or polypeptide of this invention include membrane, solution or chip-based technologies (see Hampton, R. *et al.*, (1990) Serological Methods, a Laboratory Manual, APS Press, St Paul, MN) and Maddox, D.E. *et al.*, (1983) J. Exp. Med, 158, 1211-1216).

Transgenic animals

In another embodiment of this invention, a nucleic acid molecule according to the invention may be used to create a transgenic animal, most commonly a rodent. The modification of the animal's genome may either be done locally, by modification of somatic cells or by germ line therapy to incorporate inheritable modifications. Such transgenic animals may be particularly useful in the generation of animal models for drug molecules effective as modulators of the polypeptides of the present invention.

Polypeptide purification

A polypeptide according to the invention may be recovered and purified from recombinant cell cultures by methods including, but not limited to cell lysis techniques, ammonium sulphate precipitation, ethanol precipitation, acid extraction, anion or cation chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and

lectin chromatography, high performance liquid chromatography (HPLC) or fast performance liquid chromatography (FPLC). The polypeptide may need refolding after purification or isolation and many well known techniques are available that will help regenerate an active polypeptide conformation.

Many expression vectors are commercially available that aid purification of the relevant polypeptide. These include vectors that join the sequence encoding the polypeptide to another expressed sequence creating a fused protein that is easier to purify. Ways in which these fused parts can facilitate purification of the polypeptide of this invention include fusions that can increase the solubility of the polypeptide, joining of metal chelating peptides (for example, histidine-tryptophan modules) that allow for purification with immobilised metals, joining of protein A domains which allow for purification with immobilised immunoglobulins and the joining of the domain that is utilised in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, WA). Fusion of the polypeptide of this present invention with a secretion signal polypeptide may also aid purification. This is because the medium into which the fused polypeptide has been secreted can subsequently be used to recover and purify the expressed polypeptide.

If necessary, these extraneous polypeptides often comprise a cleavable linker sequence which allows the polypeptide to be isolated from the fusion. Cleavable linker sequences between the purification domain and the polypeptide of the invention include those specific for Factor Xa or for enterokinase (Invitrogen, San Diego, CA). One such expression vector provides for expression of a fusion protein containing the polypeptide of the invention fused to several histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification by IMAC (immobilised metal ion affinity chromatography as described in Porath, J. *et al.* (1992), Prot. Exp. Purif. 3: 263-281), while the thioredoxin or enterokinase cleavage site provides a means for purifying the polypeptide from the fusion protein. A discussion of vectors that contain fusion proteins is provided in Kroll, D.J. *et al.* (1993; DNA Cell Biol. 12:441-453).

Assays

Another aspect of this invention includes assays that may be carried out using a polypeptide or nucleic acid molecule according to the invention. Such assays may be for many uses including the development of drug candidates, for diagnostic purposes or for the gathering of information for therapeutics.

If the polypeptide is to be expressed for use in screening assays, generally it is preferred that it be produced at the surface of the host cell in which it is expressed. In this event, the host cells may be harvested prior to use in the screening assay, for example using techniques such as fluorescence activated cell sorting (FACS) or immunoaffinity techniques. If the polypeptide is secreted into the medium, the

medium can be recovered in order to recover and purify the expressed polypeptide. If polypeptide is produced intracellularly, the cells must first be lysed before the polypeptide is recovered.

The polypeptide of the invention can be used to screen libraries of compounds in any of a variety of drug screening techniques. Such compounds may activate (agonise) or inhibit (antagonise) the level of expression of the gene or the activity of the polypeptide of the invention and form a further aspect of the present invention. Examples of suitable compounds are those which are effective to alter the expression of a natural gene which encodes a polypeptide of the invention or to regulate the activity of a polypeptide of the invention.

Agonist or antagonist compounds may be isolated from, for example, cells, cell-free preparations, chemical libraries or natural product mixtures. These agonists or antagonists may be natural or modified substrates, ligands, enzymes, receptors or structural or functional mimetics. For a suitable review of such screening techniques, see Coligan et al., Current Protocols in Immunology 1(2):Chapter 5 (1991).

Potential agonists or antagonists include small organic molecules, peptides, polypeptides and antibodies that bind to the polypeptide of the invention and thereby modulate its activity. In this fashion, binding of the polypeptide to normal cellular binding molecules may be potentiated or inhibited, such that the normal biological activity of the polypeptide is enhanced or prevented.

The polypeptide of the invention that is employed in such a screening technique may be free in solution, affixed to a solid support, borne on a cell surface or located intracellularly. In general, such screening procedures may involve using appropriate cells or cell membranes that express the polypeptide that are contacted with a test compound to observe binding, or stimulation or inhibition of a functional response. The functional response of the cells contacted with the test compound is then compared with control cells that were not contacted with the test compound. Such an assay may assess whether the test compound results in a signal generated by activation of the polypeptide, using an appropriate detection system. Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist in the presence of the test compound is observed.

Alternatively, simple binding assays may be used, in which the adherence of a test compound to a surface bearing the polypeptide is detected by means of a label directly or indirectly associated with the test compound or in an assay involving competition with a labelled competitor. In another embodiment, competitive drug screening assays may be used, in which neutralising antibodies that are capable of binding the polypeptide specifically compete with a test compound for binding. In this manner, the antibodies can be used to detect the presence of any test compound that possesses specific binding affinity for the polypeptide.

Assays may also be designed to detect the effect of added test compounds on the production of mRNA encoding the polypeptide in cells. For example, an ELISA may be constructed that measures secreted or cell-associated levels of polypeptide using monoclonal or polyclonal antibodies by standard methods known in the art, and this can be used to search for compounds that may inhibit or enhance the production of the polypeptide from suitably manipulated cells or tissues. The formation of binding complexes between the polypeptide and the compound being tested may then be measured.

Another technique for drug screening which may be used provides for high throughput screening of compounds having suitable binding affinity to the polypeptide of interest (see International patent application WO84/03564). In this method, large numbers of different small test compounds are synthesised on a solid substrate, which may then be reacted with the polypeptide of the invention and washed. One way of immobilising the polypeptide is to use non-neutralising antibodies. Bound polypeptide may then be detected using methods that are well known in the art. Purified polypeptide can also be coated directly onto plates for use in the aforementioned drug screening techniques.

A polypeptide according to the invention may be used to identify membrane-bound or soluble receptors, through standard receptor binding techniques that are known in the art, such as ligand binding and crosslinking assays in which the polypeptide is labelled with a radioactive isotope, is chemically modified, or is fused to a peptide sequence that facilitates its detection or purification, and incubated with a source of the putative receptor (for example, a composition of cells, cell membranes, cell supernatants, tissue extracts, or bodily fluids). The efficacy of binding may be measured using biophysical techniques such as surface plasmon resonance and spectroscopy. Binding assays may be used for the purification and cloning of the receptor, but may also identify agonists and antagonists of the polypeptide, that compete with the binding of the polypeptide to its receptor. Standard methods for conducting screening assays are well understood in the art.

A typical polypeptide-based assay might involve contacting the appropriate cell(s) or cell membrane(s) expressing the polypeptide with a test compound. In such assays, a polypeptide according to the invention may be free in solution, affixed to a solid support, borne on a cell surface or located intracellularly. Any response to the test compound, for example a binding response, a stimulation or inhibition of a functional response may then be compared with a control where the cell(s) or cell membrane(s) was/were not contacted with the test compound.

A binding response could be measured by testing for the adherence of a test compound to a surface bearing a polypeptide according to the invention. The test compound may aid polypeptide detection by being labelled, either directly or indirectly. Alternatively, the polypeptide itself may be labelled, for example, with a radioisotope, by chemical modification or as a fusion with a peptide or polypeptide

sequence that will facilitate polypeptide detection. Alternatively, a binding response may be measured, for example, by performing a competition assay with a labelled competitor or *vice versa*. One example of such a technique is a competitive drug screening assay, where neutralising antibodies that are capable of specifically binding to the polypeptide compete with a test compound for binding. In this manner, the antibodies may be used to detect the presence of any test compound that possesses specific binding affinity for the polypeptide. Alternative binding assay methods are well known in the art and include, but are not limited to, cross-linking assays and filter binding assays. The efficacy of binding may be measured using biophysical techniques including surface plasmon resonance and spectroscopy.

High throughput screening is a type of assay which enables a large number of compounds to be searched for any significant binding activity to the polypeptide of interest (see patent application WO84/03564). This is particularly useful in drug screening. In this scenario, many different small test compounds are synthesised on to a solid substrate. The polypeptide is then introduced to this substrate and the whole apparatus washed. The polypeptide is then immobilised by, for example, using non-neutralising antibodies. Bound polypeptide may then be detected using methods that are well known in the art. Purified polypeptide may also be coated directly onto plates for use in the aforementioned drug screening techniques.

Assay methods that are also included within the terms of the present invention are those that involve the use of the genes and polypeptides of the invention in overexpression or ablation assays. Such assays involve the manipulation of levels of these genes/polypeptides in cells and assessment of the impact of this manipulation event on the physiology of the manipulated cells. For example, such experiments reveal details of signaling and metabolic pathways in which the particular genes/polypeptides are implicated, generate information regarding the identities of polypeptides with which the studied polypeptides interact and provide clues as to methods by which related genes and proteins are regulated.

Another aspect of this invention provides for any screening kits that are based or developed from any of the aforementioned assays.

C. Pharmaceuticals

A further aspect of the invention provides a pharmaceutical composition suitable for modulating hypoxia and/or ischaemia, comprising a therapeutically-effective amount of a polypeptide, a nucleic acid molecule, vector or ligand as described above, in conjunction with a pharmaceutically-acceptable carrier. A composition containing a polypeptide, nucleic acid molecule, ligand or any other compound of this present invention (herein known as X) is considered to be "substantially free of impurities" (herein known as Y) when X makes up more than 85% mass per mass of the total [X+Y] mass. Preferably X comprises

at least 90% of the total X+Y mass. More preferably X comprises at least 95%, 98% and most preferably 99% of the total X+Y mass.

Carriers

Carrier molecules may be genes, polypeptides, antibodies, liposomes or indeed any other agent provided that the carrier does not itself induce toxicity effects or cause the production of antibodies that are harmful to the individual receiving the pharmaceutical composition. Further examples of known carriers include polysaccharides, polylactic acids, polyglycolic acids and inactive virus particles. Carriers may also include pharmaceutically acceptable salts such as mineral acid salts (for example, hydrochlorides, hydrobromides, phosphates, sulphates) or the salts of organic acids (for example, acetates, propionates, malonates, benzoates). Pharmaceutically acceptable carriers may additionally contain liquids such as water, saline, glycerol, ethanol or auxiliary substances such as wetting or emulsifying agents, pH buffering substances and the like. Carriers may enable the pharmaceutical compositions to be formulated into tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions to aid intake by the patient. A thorough discussion of pharmaceutically acceptable carriers is available in Remington's Pharmaceutical Sciences (Mack Pub. Co., N.J. 1991).

Dosage

The amount of component X in the composition should also be in therapeutically effective amounts. The phrase "therapeutically effective amounts" used herein refers to the amount of agent needed to treat, ameliorate, or prevent (for example, when used as a vaccine) a targeted disease or condition. An effective initial method to determine a "therapeutically effective amount" may be by carrying out cell culture assays (for example, using neoplastic cells) or using animal models (for example, mice, rabbits, dogs or pigs). In addition to determining the appropriate concentration range for X to be therapeutically effective, animal models may also yield other relevant information such as preferable routes of administration that will give maximum effectiveness. Such information may be useful as a basis for patient administration. A "patient" as used in herein refers to the subject who is receiving treatment by administration of X. Preferably, the patient is human, but the term may also include animals.

The therapeutically-effective dosage will generally be dependent on the patient's status at the time of adminstration. Factors that may be taken into consideration when determining dosage include the severity of the disease state in the patient, the general health of the patient, the age, weight, gender, diet, time and frequency of administration, drug combinations, reaction sensitivities and the patient's tolerance or response to the therapy. The precise amount can be determined by routine experimentation but may ultimately lie with the judgement of the clinician. Generally, an effective dose will be from 0.01 mg/kg (mass of drug compared to mass of patient) to 50 mg/kg, preferably 0.05 mg/kg to 10 mg/kg.

Compositions may be administered individually to a patient or may be administered in combination with other agents, drugs or hormones.

Routes of administration

Uptake of a pharmaceutical composition of the invention by a patient may be initiated by a variety of methods including, but not limited to enteral, intra-arterial, intrathecal, intramedullary, intramuscular, intranasal, intraperitoneal, intravaginal, intravenous, intraventricular, oral, rectal (for example, in the form of suppositories), subcutaneous, sublingual, transcutaneous applications (for example, see WO98/20734) or transdermal means.

Gene guns or hyposprays may also be used to administer the pharmaceutical compositions of the invention. Typically, the therapeutic compositions may be prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. Direct delivery of the compositions can generally be accomplished by injection, subcutaneously, intraperitoneally, intravenously or intramuscularly, or delivered to the interstitial space of a tissue. The compositions can also be administered into a lesion. Dosage treatment may be a single dose schedule or a multiple dose schedule.

Inhibition of excessive activity

If a particular disease state is partially or completely caused by an inappropriate excess in the activity of a polypeptide according to the invention, several approaches are available for inhibiting this activity.

One approach comprises administering to a patient an inhibitor compound (antagonist) along with a pharmaceutically acceptable carrier in an amount effective to inhibit the function of the polypeptide, such as by blocking the binding of a ligand, substrate, enzyme, receptor, or by inhibiting a second signal, and thereby alleviating the abnormal condition. Such an antagonist molecule may, for example, be an antibody. Most preferably, such antibodies are chimeric and/or humanised to minimise their immunogenicity, as previously described.

In another approach, soluble forms of the polypeptide that retain binding affinity for the ligand, substrate, enzyme, receptor, in question, may be administered to the patient to compete with the biological activity of the endogenous polypeptide. Typically, the polypeptide may be administered in the form of a fragment that retains a portion that is relevant for the desired biological activity.

In an alternative approach, expression of the gene encoding the polypeptide can be inhibited using expression blocking techniques, such as by using antisense nucleic acid molecules (as described above), either internally generated or separately administered. Modifications of gene expression may be effected by designing complementary sequences or antisense molecules (DNA, RNA, or PNA) to the control, 5' or

regulatory regions (signal sequence, promoters, enhancers and introns) of the gene encoding the polypeptide. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature (Gee, J.E. *et al.* (1994) In: Huber, B.E. and B.I. Carr, Molecular and Immunologic Approaches, Futura Publishing Co., Mt. Kisco, NY). The complementary sequence or antisense molecule may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes. Such oligonucleotides may be administered or may be generated *in situ* from expression *in vivo*.

Gene silencing approaches may also be undertaken to down-regulate endogenous expression of a gene. RNA interference (RNAi) (Elbashir, SM et al., Nature 2001, 411, 494-498) is one method of sequence specific post-transcriptional gene silencing that may be employed. Short dsRNA oligonucleotides are synthesised *in vitro* and introduced into a cell. The sequence specific binding of these dsRNA oligonucleotides triggers the degradation of target mRNA, reducing or ablating target protein expression.

In addition, expression of a polypeptide according to the invention may be prevented by using a ribozyme specific to the encoding mRNA sequence for the polypeptide. Ribozymes are catalytically active RNAs that can be natural or synthetic (see for example Usman, N, *et al.*, Curr. Opin. Struct. Biol (1996) 6(4), 527-33). Synthetic ribozymes can be designed to specifically cleave mRNAs at selected positions thereby preventing translation of the mRNAs into functional polypeptide. Ribozymes may be synthesised with a natural ribose phosphate backbone and natural bases, as normally found in RNA molecules. Alternatively the ribozymes may be synthesised with non-natural backbones, for example, 2'-O-methyl RNA, to provide protection from ribonuclease degradation and may contain modified bases.

Efficacy of the gene silencing approaches assessed above may be assessed through the measurement of polypeptide expression (for example, by Western blotting), and at the RNA level using TaqMan-based methodologies.

RNA molecules may be modified to increase their intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of non-traditional bases such as inosine, queosine and butosine, as well as acetyl-, methyl-, thio- and similarly modified forms of adenine, cytidine, guanine, thymine and uridine that are not as easily recognised by endogenous endonucleases.

Activation of a polypeptide activity

If a particular disease state is partially or completely due to a lowered level of biological activity from a polypeptide according to the invention, various methods may be used. An example of such a method includes administering a therapeutically effective amount of compound that can activate (i.e. an agonist) or cause increased expression of the polypeptide concerned. Administration of such a compound may be via any of the methods described previously.

Gene Therapy

Another aspect of the present invention provides for gene therapy methods involving nucleic acid molecules identified herein. Gene therapy may be used to affect the endogenous production of the polypeptide of the present invention by relevant cells in a patient. For example, gene therapy can be used permanently to treat the inappropriate production of a polypeptide by replacing a defective gene with the corrected therapeutic gene.

Treatment may be effected either *in vivo* or *ex vivo*. *Ex vivo* gene therapy generally involves the isolation and purification of the patient's cells, introduction of the therapeutic gene into the cells and finally, the introduction of the genetically-altered cells back into the patient. *In vivo* gene therapy does not require the isolation and purification of patient cells prior to the introduction of the therapeutic gene into the patient. Instead, the therapeutic gene can be packaged for delivery into the host. Gene delivery vehicles for *in vivo* gene therapy include, but are not limited to, non-viral vehicles such as liposomes, replication-competent and replication-deficient viruses (for example, adenovirus as described by Berkner, K.L., in Curr. Top. Microbiol. Immunol., 158, 39-66 (1992)) or adeno-associated virus (AAV) vectors as described by Muzyczka, N., in Curr. Top. Microbiol. Immunol., 158, 97-129 (1992) and U.S. Patent No. 5,252,479. Alternatively, "naked DNA" may be directly injected into the bloodstream or muscle tissue as a form of *in vivo* gene therapy.

One example of a strategy for gene therapy including a nucleic acid molecule of this present invention may be as follows. A nucleic acid molecule encoding a polypeptide of the invention is engineered for expression in a replication-defective or replication-competent vector, such as a retroviral vector. This expression construct may then be isolated and introduced into a packaging cell transduced with a retroviral plasmid vector containing RNA encoding the polypeptide, such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a patient for engineering cells *in vivo* and expression of the polypeptide *in vivo* (see Chapter 20, Gene Therapy and other Molecular Genetic-based Therapeutic Approaches, (and references cited therein) in Human Molecular Genetics (1996), T Strachan and A P Read, BIOS Scientific Publishers Ltd).

Genetic delivery of antibodies that bind to polypeptides according to the invention may also be effected, for example, as described in International patent application WO98/55607.

Vaccines

A further embodiment of the present invention provides that the polypeptides or nucleic acid molecules identified may be used in the development of vaccines. Where the aforementioned polypeptide or nucleic acid molecule is a disease-causing agent, vaccine development can involve the raising of antibodies against such agents. Where the aforementioned polypeptide or nucleic acid molecule is that is upregulated, vaccine development can involve the raising of antibodies or T cells against such agents (as described in WO00/29428).

Vaccines according to the invention may either be prophylactic (i.e. prevents infection) or therapeutic (i.e. treats disease after infection). Such vaccines comprise immunising antigen(s), immunogen(s), polypeptide(s), protein(s) or nucleic acid, usually in combination with pharmaceutically-acceptable carriers as described above. Additionally, these carriers may function as immunostimulating agents ("adjuvants"). Furthermore, the antigen or immunogen may be conjugated to a bacterial toxoid, such as a toxoid from diphtheria, tetanus, cholera, *H. pylori*, and other pathogens.

Vaccination processes may involve the use of heterologous vectors eg: prime with MVA and boost with DNA.

Since polypeptides may be broken down in the stomach, vaccines comprising polypeptides are preferably administered parenterally (for instance, subcutaneous, intramuscular, intravenous, or intradermal injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions that may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the recipient, and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents.

The vaccine formulations of the invention may be presented in unit-dose or multi-dose containers. For example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

The technology referred to as jet injection (see, for example, www.powderject.com) may also be useful in the formulation of vaccine compositions.

In accordance with this aspect of the present invention, polypeptides can be delivered by viral or non-viral techniques. Non-viral delivery systems include but are not limited to DNA transfection methods. Here, transfection includes a process using a non-viral vector to deliver a antigen gene to a target mammalian

cell. Typical transfection methods include electroporation, nucleic acid biolistics, lipid-mediated transfection, compacted nucleic acid-mediated transfection, liposomes, immunoliposomes, lipofectin, cationic agent-mediated, cationic facial amphiphiles (CFAs) (Nature Biotechnology 1996 14; 556), multivalent cations such as spermine, cationic lipids or polylysine, 1, 2,-bis (oleoyloxy)-3-(trimethylammonio) propane (DOTAP)-cholesterol complexes (Wolff and Trubetskoy 1998 Nature Biotechnology 16: 421) and combinations thereof.

Viral delivery systems include but are not limited to adenovirus vectors, adeno-associated viral (AAV) vectors, herpes viral vectors, influenza, retroviral vectors, lentiviral vectors or baculoviral vectors, venezuelan equine encephalitis virus (VEE), poxviruses such as: canarypox virus (Taylor et al 1995 Vaccine 13:539-549), entomopox virus (Li Y et al 1998 XIIth International Poxvirus Symposium p144. Abstract), penguine pox (Standard et al. J Gen Virol. 1998 79:1637-46) alphavirus, and alphavirus based DNA vectors.

In addition to the use of polypeptide-based vaccines, this aspect of the invention includes the use of genetically-based vaccines, for example, those vaccines that are effective through eliciting the expression of a particular gene (either endogenous or exogenously derived) in a cell, so targeting this cell for destruction by the immune system of the host organism.

A number of suitable methods for vaccination and vaccine delivery systems are described in International patent application WO00/29428.

D. Diagnostics

Another aspect of the present invention provides for the use of a nucleic acid molecule identified herein as a diagnostic reagent.

For example, a nucleic acid molecule may be detected or isolated from a patient's tissue and used for diagnostic purposes. "Tissue" as defined herein refers to blood, urine, any matter obtained from a tissue biopsy or any matter obtained from an autopsy. Genomic DNA from the tissue sample may be used directly for detection of a hypoxia-related condition. Alternatively, the DNA may be amplified using methods such as polymerase chain reaction (PCR), the ligase chain reaction (LCR), strand displacement amplification (SDA), or other amplification techniques (see Saiki *et al.*, Nature, 324, 163-166 (1986); Bej, *et al.*, Crit. Rev. Biochem. Molec. Biol., 26, 301-334 (1991); Birkenmeyer *et al.*, J. Virol. Meth., 35, 117-126 (1991) and Brunt, J., Bio/Technology, 8, 291-294 (1990)). Such diagnostics are particularly useful for prenatal and even neonatal testing.

A method of diagnosis of disease using a polynucleotide may comprise assessing the level of expression of the natural gene and comparing the level of encoded polypeptide to a control level measured in a

normal subject that does not suffer from the disease or physiological condition that is being tested. The diagnosis may comprise the following steps:

a) contacting a sample of tissue from the patient with a nucleic acid probe under stringent conditions that allow the formation of a hybrid complex between a nucleic acid molecule of the invention and the probe;

b) contacting a control sample with said probe under the same conditions used in step a); and

c) detecting the presence of hybrid complexes in said samples;

wherein detection of differing levels of the hybrid complex in the patient sample compared to levels of the hybrid complex in the control sample is indicative of the dysfunction.

A further aspect of the invention comprises a diagnostic method comprising the steps of:

a) obtaining a tissue sample from a patient being tested for disease;

b) isolating a nucleic acid molecule according to the invention from said tissue sample; and

c) diagnosing the patient for disease by detecting the presence of a mutation in the nucleic acid molecule which is associated with disease.

To aid the detection of nucleic acid molecules in the above-described methods, an amplification step, such as PCR, may be included. An example of this includes detection of deletions or insertions indicative of the dysfunction by a change in the size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridising amplified DNA to labelled RNA of the invention or alternatively, labelled antisense DNA sequences of the invention. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase digestion or by assessing differences in melting temperatures. The presence or absence of the mutation in the patient may be detected by contacting DNA with a nucleic acid probe that hybridises to the DNA under stringent conditions to form a hybrid double-stranded molecule, the hybrid double-stranded molecule having an unhybridised portion of the nucleic acid probe strand at any portion corresponding to a mutation associated with disease; and detecting the presence or absence of an unhybridised portion of the probe strand as an indication of the presence or absence of a disease-associated mutation in the corresponding portion of the DNA strand.

Point mutations and other sequence differences between the reference gene and "mutant" genes can be identified by other well-known techniques, such as direct DNA sequencing or single-strand conformational polymorphism, (see Orita *et al.*, Genomics, 5, 874-879 (1989)). For example, a sequencing primer may be used with double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence determination is performed by conventional procedures with radiolabelled nucleotides or by automatic sequencing procedures with fluorescent-tags.

Cloned DNA segments may also be used as probes to detect specific DNA segments. The sensitivity of this method is greatly enhanced when combined with PCR. Further, point mutations and other sequence variations, such as polymorphisms, can be detected as described above, for example, through the use of allele-specific oligonucleotides for PCR amplification of sequences that differ by single nucleotides.

DNA sequence differences may also be detected by alterations in the electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing (for example, Myers *et al.*, Science (1985) 230:1242). Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method (see Cotton *et al.*, PNAS. USA (1985) 85: 4397-4401).

In addition to conventional gel electrophoresis and DNA sequencing, mutations such as microdeletions, aneuploidies, translocations, inversions, can also be detected by *in situ* analysis (see, for example, Keller *et al.*, DNA Probes, 2nd Ed., Stockton Press, New York, N.Y., USA (1993)), that is, DNA or RNA sequences in cells can be analysed for mutations without need for their isolation and/or immobilisation onto a membrane. FISH is presently the most commonly applied method and numerous reviews of FISH have appeared (see, for example, Trachuck *et al.*, Science, 250, 559-562 (1990), and Trask *et al.*, Trends, Genet., 7, 149-154 (1991)).

Arrays

In another embodiment of the invention, an array of oligonucleotide probes comprising a nucleic acid molecule according to the invention can be constructed to conduct efficient screening of genetic variants, mutations and polymorphisms. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability (see for example: M.Chee *et al.*, Science (1996), Vol 274, pp 610-613).

In one embodiment, the array is prepared and used according to the methods described in WO95/11995 (Chee *et al*); Lockhart, D. J. *et al.* (1996) Nat. Biotech. 14: 1675-1680); and Schena, M. *et al.* (1996) PNAS 93: 10614-10619). Oligonucleotide pairs may range from two to over one million. The oligomers are synthesized at designated areas on a substrate using a light-directed chemical process. The substrate may be paper, nylon or other type of membrane, filter, chip, glass slide or any other suitable solid support. In another aspect, an oligonucleotide may be synthesized on the surface of the substrate by using a chemical coupling procedure and an ink jet application apparatus, as described in PCT application W095/251116 (Baldeschweiler *et al*). In another aspect, a "gridded" array analogous to a dot (or slot) blot may be used to arrange and link cDNA fragments or oligonucleotides to the surface of a substrate using a vacuum system, thermal, UV, mechanical or chemical bonding procedures. An array, such as those described above, may be produced by hand or by using available devices (slot blot or dot blot apparatus),

materials (any suitable solid support), and machines (including robotic instruments), and may contain 8, 24, 96, 384, 1536 or 6144 oligonucleotides, or any other number between two and over one million which lends itself to the efficient use of commercially-available instrumentation.

Diagnostics using polypeptides or mRNA

In addition to the methods discussed above, diseases may be diagnosed by methods comprising determining, from a sample derived from a subject, an abnormally decreased or increased level of polypeptide or mRNA. Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the quantitation of polynucleotides, such as, for example, nucleic acid amplification, for instance PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods.

Assay techniques that can be used to determine levels of a polypeptide of the present invention in a sample derived from a host are well-known to those of skill in the art and are discussed in some detail above (including radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays). One example of this aspect of the invention provides a diagnostic method which comprises the steps of: (a) contacting a ligand as described above with a biological sample under conditions suitable for the formation of a ligand-polypeptide complex; and (b) detecting said complex.

Protocols such as ELISA, RIA, and FACS for measuring polypeptide levels may additionally provide a basis for diagnosing altered or abnormal levels of polypeptide expression. Normal or standard values for polypeptide expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, preferably humans, with antibody to the polypeptide under conditions suitable for complex formation The amount of standard complex formation may be quantified by various methods, such as by photometric means.

Antibodies which specifically bind to a polypeptide of the invention may be used for the diagnosis of conditions or diseases characterised by expression of the polypeptide, or in assays to monitor patients being treated with the polypeptides, nucleic acid molecules, ligands and other compounds of the invention. Antibodies useful for diagnostic purposes may be prepared in the same manner as those described above for therapeutics. Diagnostic assays for the polypeptide include methods that utilise the antibody and a label to detect the polypeptide in human body fluids or extracts of cells or tissues. The antibodies may be used with or without modification, and may be labelled by joining them, either covalently or non-covalently, with a reporter molecule. A wide variety of reporter molecules known in the art may be used, several of which are described above.

Quantities of polypeptide expressed in subject, control and disease samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the

parameters for diagnosing disease. Diagnostic assays may be used to distinguish between absence, presence, and excess expression of polypeptide and to monitor regulation of polypeptide levels during therapeutic intervention. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal_studies, in clinical trials or in monitoring the treatment of an individual patient.

Diagnostic kits

A diagnostic kit of the present invention may comprise:

(a) a nucleic acid molecule of the present invention;

(b) a polypeptide of the present invention; or

(c) a ligand of the present invention.

In one aspect of the invention, a diagnostic kit may comprise a first container containing a nucleic acid probe that hybridises under stringent conditions with a nucleic acid molecule according to the invention; a second container containing primers useful for amplifying the nucleic acid molecule; and instructions for using the probe and primers for facilitating the diagnosis of disease. The kit may further comprise a third container holding an agent for digesting unhybridised RNA.

In an alternative aspect of the invention, a diagnostic kit may comprise an array of nucleic acid molecules, an array of antibody molecules, and/or an array of polypeptide molecules, as discussed in more detail above.

Such kits will be of use in diagnosing a disease or susceptibility to disease, particularly inflammation, oncology, or cardiovascular disease.

Various aspects and embodiments of the present invention will now be described in more detail by way of example, with particular reference to polypeptides regulated differentially under hypoxic conditions as opposed to normoxic conditions. It will be appreciated that modification of detail may be made without departing from the scope of the invention.

Brief description of the Figures

Figure 1 shows a scatter plot, showing normalised signal intensities in hypoxia versus normoxia, with each dot representing a single gene.

Figure 2: Hypoxia responses amplified by HIF1alpha overexpression. Data shown is the average of 4 repeat experiments. Values represent fold change as compared to untreated cells (condition 1). Error bars represent standard error of the mean.

Figure 3: Hypoxia responses amplified by EPAS1 overexpression. Data shown is the average of 4 repeat experiments. Values represent fold change as compared to untreated cells (condition 1). Error bars represent standard error of the mean.

Figure 4: Hypoxia responses amplified by HIF1alpha / EPAS1 overexpression. Data shown is the average of 4 repeat experiments. Values represent fold change as compared to untreated cells (condition 1). Error bars represent standard error of the mean.

Figure 5 shows genes that are induced by hypoxia to a greater degree in resting macrophages, as compared to activated macrophages. Error bars show the standard deviation from both repeat experiments and multiple exposures from single experiments. These data are not shown in table form. All bars are ratios of mRNA expression in hypoxia/ normoxia. These are calculated separately for resting (light bars) and activated (dark bars) macrophages, and do not illustrate differences resulting from activation in normoxia.

Figure 6 shows genes which are induced by hypoxia to a greater degree in activated macrophages, compared to resting macrophages.

Figure 7 shows genes that are repressed by hypoxia to a greater degree in activated macrophages.

For Figures 8, 9a, 9c, 10-32a, 32d and 33-52, mRNA levels, determined from a custom gene array, of particular genes are shown on the Y-axis, expressed as a value as compared to the median expression level of this gene throughout all samples. Eleven primary human cell types as shown on the x-axis were cultured in normoxia (black), or exposed to hyopxia for 6hr (grey) or 18hr (white).

Figure 8: Ecotropic viral integration site 2A (Seq ID:475/476).

Figure 9a: Novel PI-3-kinase adapter (Seq ID:79/80); Image clone accession R62339.

Figure 9b: TaqMan Real-time Q-RT-PCR data for Novel PI-3-kinase adapter (Seq ID:79/80); Image clone accession R62339.

Figure 9c: IMAGE clone acc R59598 (Syk).

Figure 10: Regulator of G-protein signalling 1 (Seq ID:375/376)

Figure 11: GM2 ganglioside activator protein (Seq ID:389/390)

Figure 12: Hypothetical protein PRO0823 (Seq ID:21/22)

Figure 13: CYP1 (cytochrome P450, subfamily XXVIIB) (Seq ID:339/340)

Figure 14: Alpha-2-macroglobulin (Seq ID:405/406)

Figure 15: Interleukin 1 receptor antagonist (Seq ID:357/358)

Figure 16: SCYA3L (Seq ID:469/470)

Figure 17: CFFM4 (Seq ID:433/434)

Figure 18: Pleckstrin (Seq ID:431/432)

Figure 19: CYP1B1 (SeqID:325/326)

Figure 20: CYP1B1 (SeqID:137/138)

Figure 21: Hypothetical protein FLJ13511 (SeqID:163/164)

Figure 22: Hematopoietic Zinc finger protein (SeqID:17/18)

Figure 23: Osteopontin (SeqID:267/268)

Figure 24: Osteopontin (SeqID:267/268)

Figure 25: Adipophilin (SeqID:313/314)

Figure 26: Adipophilin (SeqID:313/314)

Figure 27: Adipophilin (SeqID:313/314)

Figure 28: Adipophilin (SeqID:313/314)

Figure 29: Hypothetical protein FLJ22690 (SeqID:205/206)

Figure 30: cDNA DKFZp586E1624 (SeqID: 65/66)

Figure 31: EST (SeqID:197/198)

Figure 32a: EGL nine (C.elegans) homolog 3 (SeqID:85/86)

Figure 32b: Gene expression profiles in macrophages with and without activation. mRNA levels, determined from a custom gene array, of c1orf12 are shown on the Y-axis, expressed as a value compared to the mean value of a set of control genes on each array (per-chip normalisation). All cells were human macrophages, cultured either without cytokines or with IL-10 or with the combination of IFN□ and LPS in normoxia and hypoxia.

Figure 32c: Gene expression profiles in macrophages with and without activation. mRNA levels, determined from a custom gene array, of EGLN3 are shown on the Y-axis, expressed as a value compared to the mean value of a set of control genes on each array (per-chip normalisation). All cells were human macrophages, cultured either without cytokines or with IL-10 or with the combination of IFN□ and LPS in normoxia and hypoxia.

Figure 32d: C1orf12 (SeqID: 89.90)

Figure 32e: The effect of EPAS/ HIF overexpression on expression of the gene C1orf12 EGLN genes using a custom gene array. mRNA expression levels of the gene c1ORF12 as determined by the custom array, in response to hypoxia and adenoviral over-expression of HIF or EPAS are shown. Experimental conditions are as follows: #1 no adeno / normoxia; #2 empty adeno (low dose)/ normoxia; #3 empty adeno (high dose)/ normoxia; #4 empty adeno (low dose)/ hypoxia; #5 empty adeno (high dose)/ hypoxia; #6 HIF-1 adeno (low dose)/ hypoxia; #7 HIF-1 adeno (high dose)/ hypoxia; #8 EPAS adeno (low dose)/ hypoxia; #9 EPAS adeno (high dose)/ hypoxia. Error bars are the standard error of the mean.

Figure 32f: The effect of EPAS/ HIF overexpression on expression of the gene EGLN3 gene using a custom gene array. mRNA expression levels of the gene EGLN3 as determined by the custom array, in response to hypoxia and adenoviral over-expression of HIF or EPAS are shown. Experimental conditions are as follows: #1 no adeno / normoxia; #2 empty adeno (low dose)/ normoxia; #3 empty adeno (high dose)/ normoxia; #4 empty adeno (low dose)/ hypoxia; #5 empty adeno (high dose)/ hypoxia; #6 HIF-1 adeno (low dose)/ hypoxia; #7 HIF-1 adeno (high dose)/ hypoxia; #8 EPAS adeno (low dose)/ hypoxia; #9 EPAS adeno (high dose)/ hypoxia. Error bars are the standard error of the mean.

Figure 32g: The effect of EPAS/ HIF overexpression on expression of the EGLN3 gene using AffyMetrix Hu95 ver2 GeneChips. mRNA expression levels of the gene in response to hypoxia and adenoviral over-expression of HIF or EPAS are shown. Graphs show the mean of two replicate arrays, with error bars as standard deviation. Above each graph, data values are shown, including the normalised values and raw values (the AffyMetrix average difference parameter) and Present/ Absent flags.

Figure 32h: The effect of EPAS/ HIF overexpression on expression of the c1orf12 gene using AffyMetrix Hu95 ver2 GeneChips. mRNA expression levels of the gene in response to hypoxia and adenoviral over-expression of HIF or EPAS are shown. Graphs show the mean of two replicate arrays, with error bars as standard deviation. Above each graph, data values are shown, including the normalised values and raw values (the AffyMetrix average difference parameter) and Present/ Absent flags.

Figure 32i: Flag immunocytochemistry in HEK293T cells

Figure 32j: Human Cardiomyocyte Caspase Activity after 72 hours transduction with EIAV-ELG9-Homolog 3

Figure 33: Novel Metallothionein (SeqID:83/84)

Figure 34: Hypothetical protein hqp0376 (SeqID:337/338)

Figure 35: Metallothionein 2A (SeqID:265/266)

Figure 36: Metallothionein 1G (SeqID:243/244)

Figure 37: Metallothionein 1H (SeqID: 239/240)

Figure 38: Hepcidin antimicrobial peptide (SeqID:141/142)

Figure 39: EST (SeqID: 117/118)

Figure 40: Hypothetical protein FLJ22622 (SeqID:129/130)

Figure 41: TRIP-Br2 (SeqID:31/32)

Figure 42: Tumor protein D52 (SeqID:301/302)

Figure 43: Semaphorin 4b (SeqID:91/92/92a)

Figure 44: Dec-1 (SeqID:371/372)

Figure 45: Calgranulin A (SeqID:447/448)

Figure 46: ERO1 (S. cerevisiae)-like (SeqID:67/68)

Figure 47: Hypothetical protein FLJ20500 (SeqID:25/26)

Figure 48: N-myc downstream regulated (SeqID:229/230)

Figure 49: Decidual protein induced by progesterone (SeqID:387/388)

Figure 50: Integrin, alpha 5 (SeqID:379/380)

Figure 51: Tissue factor (SeqID:225/226)

Figure 52: COX-2 (SeqID:237/238)

Figure 53: Genes up-regulated by macrophage activation. Normalised mRNA levels in the 6 experimental conditions (#1 no cytokines/ normoxia, #2 no cytokines/ hypoxia, #3 IL-10/ normoxia, #4 IL-10/ hypoxia, #5 LPS/IFN/ normoxia, #6 LPS/IFN/ hypoxia) are shown as values referenced to the median value of that gene throughout all 6 experimental conditions. Error bars show the standard error of the mean.

Figure 54: Genes downregulated by macrophage activation (I)

Figure 55: Genes downregulated by macrophage activation (II)

Figure 56: Genes downregulated by macrophage activation (III)

Figure 57 shows an RNase protection assay for the gene encoding Semaphorin 4b.

Figure 58 shows a Northern blot showing the size of the mRNA and tissue distribution for the Semaphorin 4b gene.

Examples

Summary

Subtracted cDNA libraries were separately prepared for hypoxic macrophages and cardiomyoblasts. This involved harvesting RNA from cells both in normoxia and hypoxia, and preparing cDNA. Subtractive hybridization / suppression PCR was then performed to remove genes from the hypoxic cell cDNA, which are also present in cDNA from normoxic cells. Insert DNA from the libraries was PCR amplified and arrayed onto duplicate membranes. Quantitative hybridizations with pre-library cDNA material (normoxia and hypoxia) were done to identify clones in the libraries that actually contain hypoxia inducible genes. The insert DNA was then sequenced.

This procedure was done independently for macrophage and cardiomyoblast. The hypoxia inducible genes identified from these different cell types differed widely, with only a minority of these genes being identified from both cell types.

To characterise the differences between the two tissues further, arrays were produced containing all confirmed hypoxia-inducible genes from the macrophage library. Replicate arrays were hybridised with cDNA from normoxic and hypoxic cardiomyoblasts to allow quantitative evaluation of these genes in the cardiomyoblast. This revealed quantitative differences in the hypoxia induced activation these genes in the two cell types.

Example 1a: Comparison of the hypoxic-response between human macrophages and cardiomyoblasts by a subtraction cloning / array screening approach

Methods / Results

To isolate human macrophage, monocytes were derived from peripheral blood of healthy human donors. 100ml bags of buffy coat from the Bristol Blood Transfusion Centre were mixed with an equal volume of RPMI1640 medium (Sigma). This was layered on top of 10ml ficol-paque (Pharmacia) in 50ml centrifuge tubes and centrifuged for 25 min at 800 x g. The interphase layer was removed, washed in MACS buffer (phosphate buffered saline pH 7.2, 0.5% bovine serum albumin, 2mM EDTA) and resuspended at 80 microliter per 10n7 cells. To this 20 microliter CD14 Microbeads (Miltenyi Biotec) were added, and the tube incubated at 4 degrees for 15 min. Following this one wash was performed in MACS buffer at 400 x g and the cells were resuspended in 3 ml MACS buffer and separated on an LS+ MACS Separation Column (Miltenyi Biotec) positioned on a midi-MACS magnet (Miltenyi Biotec). The column was washed with 3 x 3ml MACS buffer. The column was removed from the magnet and cells were eluted in 5 ml MACS buffer using a syringe. Cells were washed in culture medium (AIM V (Sigma) supplemented with 2% human AB serum (Sigma), and resuspended at 2 x 10n5 cells per ml in the same medium and

placed in large teflon-coated culture bags (Sud-Laborbedarf GmbH, 82131 Gauting, Germany) and transferred to a tissue culture incubator (37 degrees, 5% CO2) for 7-10 days. During this period monocytes spontaneously differentiate to macrophages. This is confirmed by examining cell morphology using phase contrast microscopy. Cells are removed from the bags by placing at 4 degrees for 30 min and emptying the contents. The cells are then washed and resuspended in culture medium at 5 x 105 cell/ml and plated out in Primeria 10 cm tissue culture petri dishes (Falcon Becton Dickinson) at 5 x 10n6 cells per dish. Culture is continued for 16-24hr to allow cell adherence, prior to experimentation involving hypoxia.

As an alternative primary cell type human cardiomyoblast cultures were established. Cells derived from the ventricular tissue of newborn or foetal hearts were purchased from BioWhittaker (CC-2582). Growth conditions were used to allow maximum expansion of the cells in vitro, by using a medium rich in growth factors. Under such conditions cardiomyoblast-like cells predominate (the developmental precursor of cardiomyocytes). This has been previously described by Goldman and Wurzel (*In Vitro Cell. Dev. Biol.* **28A:** 109-119 (1992)) and Goldman *et al.*, (1996, *Exp.Cell.Res.* **228**(2): 237-245).

For these cultures, cells were seeded at $1x10^6$ per T150 flask in human smooth muscle growth medium (TCS CellWorks ZHM-3935) and were expanded in the same medium up to a maximum number of 4 passages. The growth medium is purchased pre-prepared, and includes in the formula, 5% fetal bovine serum, insulin, epidermal growth factor and fibroblast growth factor. Prior to experimentation involving hypoxia, cells were plated onto 10 cm tissue culture petri dishes and allowed to reach confluency.

For experimentation with hypoxia, for all cell types, an equal number of identical culture dishes were divided into two separate incubators: One at 37 degrees, 5% CO2, 95% air (=Normoxia) and the other at 37 degrees, 5% CO_2, 94.9% Nitrogen, 0.1% Oxygen (=Hypoxia). After 6 hours culture under these conditions, the dishes were removed from the incubator, placed on a chilled platform, washed in cold PBS and total RNA was extracted using RNazol B (Tel-Test, Inc; distributed by Biogenesis Ltd) following the manufacturer's instructions. Polyadenylated mRNA was extracted from the total RNA using a commercial kit following the manufacturer's instructions (Promega; PolyATract mRNA isolation System IV).

The hypoxia period of 6 hr was previously determined to be sufficient to allow the induction of known hypoxia-regulated genes, as determined by RNase protection assays. During these preliminary studies it was noted that macrophages, cardiomyoblasts and an additional control cell type, Jurkat T-cells, showed different patterns of gene induction in response to hypoxia:

Known Hypoxia-inducible gene	level of hypoxia-induced increase in mRNA levels		
	Macrophage	**Myoblast**	**T-cell**

phosphoglycerate kinase-1 (PGK)	none	none	high
vascular endothelial growth factor-A (VEGF)	high	low	high
solute carrier family 2, member 1 (Glut-1)	high	low	high

Separate subtracted cDNA populations were generated from mRNA extracted from hypoxic macrophages and hypoxic cardiomyoblasts, using a combination of two kits, purchased from Clontech Laboratories- SMART PCR cDNA synthesis kit and PCR Select cDNA subtraction kit. The manufacturer's instructions were followed for both kits. All diagnostic steps were followed as recommended by the manufacturers. All PCR reactions were done using an Applied Biosystems 9700 with 96-well block, using Applied Biosystems plastics. Driver and tester populations used for subtraction were as below:

subtracted cDNA	**tester**	**driver**
Subtracted macrophage	macrophage (hypoxia)	macrophage (normoxia)
Subtracted cardiomyoblast	cardiomyoblast (hypoxia)	cardiomyoblast (normoxia)

The final subtracted cDNA samples were evaluated by performing RT-PCR using the following primers for human beta actin:

sense: TCACCCACACTGTGCCCATCTACGA

antisense: CAGCGGAACCGCTCATTGCCAAATGG

This showed that an additional 5 cycles of PCR were required to achieve similar levels of beta actin product from subtracted compared to unsubtracted cDNA, indicating a significant reduction in the representation of a non-regulated gene in the subtracted cDNA. Glyceraldehyde 3-Phosphate dehydrogenase PCR primers, as contained in the kit, were not used.

The three subtracted cDNA populations were ligated into a plasmid vector (pCRII, Invitrogen) to generate libraries, which were transformed into *E.coli* (INVαF', Invitrogen) and plated out onto agar, supplemented with ampicillin and X-Gal, according to standard methods.

Colonies that are white indicate the presence of a recombinant plasmid, and these were picked into individual wells of 96-well plates containing 100 microliters LB-Ampicillin, and given 3-8 hr growth at 37 degrees. In this way, for each library, up to 15 x 96-well plates of clones were generated.

To screen clones for the presence of differentially expressed genes, replicate arrays of plasmid insert DNA were generated on nylon membranes: Firstly, PCR was performed using nested PCR primers 2R and 1, which flank the cDNA insert of each clone (sequence described in the PCR Select kit). The reaction mix also contains 200 uM d(A,T,C,G)TP, Advantage2 polymerase mix (Clontech Laboratories) and supplied 10x buffer. 40 ul reactions were set up in 96-well PCR reaction plates and inoculated with 0.5 ul bacteria from the library plates. 23 cycles of PCR were performed (95 degrees 10 sec; 68 degrees 2 min), and a selection of wells were checked on an agarose gel. In this manner a 96-well plate of insert DNA was generated for each 96-well plate of bacterial clones. Arrays of insert DNA were generated by transferring 4ul of each well to 384-well plates (Genetix), and denaturing the DNA by adding 4ul 0.4M NaOH and incubating at 37 degrees for 15 minutes. Bromophenol blue was added to the wells to allow visualisation of arraying. A 384-pin replicator (Genetix) was used to spot small volumes of denatured insert DNA onto dry nylon membranes (Hybond N+, AmershamPharmacia).

By repeating this operation from the same 384-well plate onto several membranes, matched pairs of membranes were produced, suitable for array screening. A fragment of the beta actin gene was spotted at specific positions of the arrays. Following spotting, the membranes were left at room temperature for 2 hr, re-denatured by placing on chromatography paper wetted with 0.3 M NaOH, neutralised by placing on chromatography paper wetted with 0.5 M Tris pH 7.5, dried at room temperature for 2 hr and crosslinked by exposing to 2000 joules UV radiation. Prior to hybridisation, residual salts were removed from the arrays, by washing in hot 0.5% SDS.

Matched pairs of membranes were hybridised with subtracted cDNA samples; from hypoxic and normoxic cells, to determine the abundance of the genes corresponding to each spotted clone in the cDNA samples. Because the cDNA probes were subtracted, large differences in the hybridisation signal for individual spots were apparent, which can be identified by eye. Prior to probe labelling, subtracted cDNA samples were digested with RsaI and run through Qiagen Qiaquick PCR purification columns to remove adapter sequences added during the PCR Select procedure. 25 ng cDNA was labelled with 33P using a commercial kit following the manufacturer's instructions (Promega, Prime-a-gene kit), and unincorporated label was removed using BioRad Biospin-6 columns following adding 2.5ug yeast tRNA carrier.

Pre-hybridisation, hybridisation and washes were performed essentially according to the Research Genetics GeneFilters protocol, but supplementing the hybridisation mixture with 10 ug of a cocktail of oligonucleotides complementary to the Clontech PCR Select nested PCR primers (equimolar mix of primers 1 and 2R and their reverse complements).

Hybridized arrays were exposed to X-ray film or were exposed to a phosphorimager (Molecular Dynamics, Storm) and clones showing gross differences in the hybridization signals with hypoxic compared to normoxic cDNA probes were identified. This procedure was used to process all clones originally picked from the primary libraries and PCR amplified. The selected clones were grouped together onto a single array (referred to here as a secondary array), and were re-screened with cDNA probes which had not been subtracted, to allow a more quantitative though less sensitive, evaluation of the relative abundance of the genes in hypoxia vs. normoxia.

In this case, probes were ds cDNA generated from the Clontech SMART cDNA synthesis kit (labelled using the Promega Prime-a-gene kit) or were total RNA (labelled according to the Research Genetics GeneFilters protocol), and hybridisations were done according to the Research Genetics GeneFilters protocol.

Hybridization signals were measured using a phosphorimager and were processed with ArrayVision (Imaging Research Inc) software using multiple beta-actin spots to normalise the quantitation and individual spot background correction. At this stage, the inserts of clones showing consistent up-regulation in hypoxia were sequenced using the 2R primer.

The identity of the genes were determined using BLAST at the NCBI (NLM, NIH) against the non-redundant data base collection. Where significant matches to human genes were not made, the human EST database was used. For both EST and non-EST hits, identifier numbers were also obtained from the UniGene database.

The above strategy was used independently for libraries derived from macrophages and from cardiomyoblasts. By screening a relatively large number of clones (several thousand per library), single genes were identified from multiple clones from any individual library. Multiple clones covered either the same or different regions of the genes.

In the above manner, certain hypoxia-inducible genes were identified from clones **only** derived from the cardiomyoblast library. These genes are listed in Table 1. Certain hypoxia-inducible genes were identified from clones **only** derived from the macrophage libraries. These genes are listed in Table 2. Certain hypoxia-inducible genes were identified from clones derived from **both** macrophage and myoblast libraries. These genes are listed in Table 3.

It can be seen that Table 3 contains many less genes than either Tables 1 and 2; demonstrating that these cell types have large differences in the genes induced by hypoxia. Importantly, the subtracted libraries for macrophage and cardiomyoblast were constructed in parallel. Therefore, major differences in the spectrum of genes isolated from these libraries are likely to be due to differences in the starting material, rather than due to technical differences in the production of the libraries. Importantly, the genes contained

in these tables were confirmed to be hypoxia-regulated in the relevant cell type(s) by the described two-stage array hybridisation screening process.

From Table 3 it is clear that although this subset of genes was found in subtracted libraries from both hypoxic macrophages and cardiomyoblasts, the fold-induction obtained between hypoxia and normoxia, for the different tissues differs widely. For the first 5 genes in this table, the hypoxia response is greater for macrophages, whereas for the last 2 genes it is greater for cardiomyoblasts.

To test whether genes isolated only in the macrophage-derived subtracted libraries are not responsive to hypoxia in cardiomyoblast, cardiomyoblast cDNA isolated from normoxic and hypoxic cells was hybridised to an array of macrophage-derived clones. These data are presented as a scatter plot, showing normalised signal intensities in hypoxia versus normoxia, with each dot representing a single gene on the array. This plot is presented in Figure 1. A gene that is not affected by hypoxia will localise around the y=x line, running diagonally through the centre of the graph. From the figure, it can be seen that most genes lie in this region, even though all the genes were responsive to hypoxia in the macrophage (Table 2). There is a subset of genes that lie beneath this region (x>y), representing induction of these genes by hypoxia in the cardiomyoblast.

Sequence data for the cDNA inserts of clones from our custom subtracted cDNA libraries is available. These are usually short fragments of 300-1000 bp. Some have been resequenced to obtain an accurate full insert sequence (see document "gene sequences/analysis").

Several of the genes presented in Tables 1-3 encode hypothetical proteins of unknown function and others have no database matches with protein coding sequence. The work presented here provides some functional annotation for these genes, and potential applications for the treatment of disease. Certain genes, in particular the glycolytic enzymes and transporters, have been hypothesised previously as forming part of the generic hypoxia response. The data provided herein provide solid, validating data for these hypotheses.

It was surprising to note that cells from our cultures of human ventricle-derived cells, showing a cardiomyoblast-like phenotype, do not support significant induction of the following genes: Lactate dehydrogenase A,, Enolase 1, Phosphoglycerate kinase 1, Triosephosphate isomerase 1. These genes have been identified as being targets of the "ubiquitous" transcription factor HIF-1 alpha ("HIF-1: mediator of physiological and pathophsiological responses to hypoxia" *J.Appl.Physiol* **88:** 1474-1480 (2000)).

Example 1b: Preparation of custom array

To confirm the findings presented in Example 1a, and to obtain more accurate and additional data, both the subtracted cDNA library clones and the IMAGE clones identified from the Research Genetics Human GeneFilters have now been fabricated by the authors into an independently produced and verified gene

array (referred to herein as the "custom gene array"), composed of PCR-amplified insert DNA. The methods used to produce this array are common in the art, but the key points are summarised below.

Clones from the subtracted cDNA library were PCR amplified as defined in Example 1a. In many cases, there were multiple cDNA clones corresponding to different regions of the same gene, and all these were represented on the custom gene array. IMAGE clones were obtained from the UK MRC HGMP Resource Centre (Hinxton, Cambridge CB10 1SB, UK) and were re-isolated as individual colonies and sequenced to verify the correct identity of the clone. In the majority of cases, the same IMAGE clone identified from the Research Genetics Human GeneFilters was selected, but in some instances these clones were not available and alternatives were selected, corresponding to the same gene.

Additional genes, with well-defined roles in various disease processes relevant to hypoxia, were also represented on the array, as derived from IMAGE clones. It is well established in the literature that genes with similar functions are often co-regulated at the mRNA level, as determined by microarray data clustering methods (Iyer VR *et al, Science*. 1999 283(5398):83-7; Eisen MB *et al Proc Natl Acad Sci U S A*. 1998 95(25):14863-8). This allows associations to be made between genes of unknown function (as present in the current specification) to genes of well defined function, in order to add significance to the former.

Normalisation is a key issue in array analysis. The custom gene array is a single colour type array, and contains a selection of additional IMAGE clones corresponding to genes which were empirically determined not to be affected by hypoxia and which are highly expressed in a wide range of human tissues and cell types. During data analysis, spot intensities were divided by the mean of all the reference genes shown below, each of which was present in quadruplicate on each array.

Gene	**IMAGE clone Acc.**
FLJ11102 fis clone PLACE1005646	AA464704
matrix Gla protein	AA155913
guanine nucleotide binding protein alpha stimulating 1	R43581
DKFZp434A1319	W74725
cDNA FLJ23280 fis clone HEP07194	AA669443
beta actin	(in house clone)
EF1a-like protein	AI817566
ribosomal protein L37a	W91881

IMAGE clone plasmid miniprep DNA was prepared and PCR amplified with flanking vector primers of the sequences GTTTTCCCAGTCACGACGTTG and TGAGCGGATAACAATTTCACACAG. This was

then purified and concentrated by ethanol precipitation, and the presence of a single band and DNA concentration were determined by agarose gel electrophoresis and by digital imaging methods.

Purified PCR product corresponding to all the clones (IMAGE and non-IMAGE) were normalised to 0.5 mg/ ml by dilution. Arrays were fabricated onto Hybond N+ (Amersham) membranes using a BioRobotics TAS arrayer (Biorobotics, Cambridge CB37LW, UK) with a 500 micron pin tool. Using 384-well source plates and a 2x2 arraying format this array was relatively low density, thereby eliminating problems of spot-to-spot signal bleed. Also the large pin size and high source plate DNA concentration improves the sensitivity of detection. Post-arraying denaturation/ neutralisation was essentially as described by Bertucci F *et al.*, 1999 (*Oncogene* 18: 3905-3912).

Total RNA was extracted from cells using RNeasy (Qiagen) and 7 micrograms RNA was labelled with 100 microCi 33P dCTP using 2 micrograms poly dT (10-20 mer) as primer in a reverse transcription reaction. First strand RNA was then degraded under alkaline contitions, and this was then neutralised with Tris HCl pH 8.0, and the labelled cDNA was purified using BioRad BioSpin-6 chromatography columns. Pre-hybridisation was performed in 4 ml Research Genetics MicroHyb solution supplemented with 10micrograms poly dA (10-20 mer) and 10 micrograms Cot-1 DNA, at 45 degrees for 2-3 hours. The cDNA was then denatured by heating and added to the pre-hybridisation, which was continued for 18-20hr. Washing steps were done as follows: 2xSSC/ 1% SDS 2x20min at 50 degrees and 0.5xSSC/ 1% SDS 10min at 55 degrees. Arrays were exposed to Amersham Low Energy phosphor screens for 24hr and scanned using a phosphorimager at 50 micron resolution. Image analysis was done using ArrayVision software (Imaging Research Inc). Tab delimited data files were exported and a full analysis performed using GeneSpring software (Silicon Genetics).

Using the described methodology a dynamic range of detection of 4 logs and a sensitivity of at least 1 / 50,000 is obtained, as determined by spike doping titration experiments. Having several technical differences compared to the Research Genetics Human GeneFilters as used in the initial filing, data from the custom gene array is expected to be quantitatively different.

Example 1c: Hypoxia regulation of gene expression in macrophages by exposing cells to hypoxia +/- additional signal amplification.

The transcription factor HIF-1α, is ubiquitously present in cells and is responsible for the induction of a number of genes in response to hypoxia. This protein is considered a master regulator of oxygen homeostasis (see, for example, Semenza, (1998) Curr. Op. Genetics and Dev. 8:588-594). Although HIF-1a is well known to mediate responses to hypoxia, other transcription factors are also known or suspected to be involved. These include a protein called endothelial PAS domain protein 1 (EPAS1) or HIF-2a, which shares 48% sequence identity with HIF-1a (Tian H, et al. *Genes Dev.* 1997 11:72-82.). Evidence

suggests that EPAS1 is especially important in mediating the hypoxia-response in certain cell types, and it is clearly detectable in human macrophages, suggesting a role in this cell type (Griffiths et al., 2000, Gene Ther., 7(3):255-62).

As supporting evidence for the hypoxic regulation of the genes contained within this specification, adenoviral vectors were used to overexpress HIF-1a and EPAS1 in primary human macrophages prior to exposure to hypoxia, in order to amplify the response. Because the role of these transcription factors as mediators of the hypoxia response is very well established, any further increases in the inducibility of specific genes resulting from this approach represents credible supporting evidence that those genes are responsive to hypoxia.

A commercially available system was used herein to produce adenoviral particles involving the adenoviral transfer vector AdApt, the adenoviral genome plasmid AdEasy and the packaging cell line Per-c6 (Crucell, Leiden, The Netherlands). The standard manufacturer's instructions were followed. Three derivatives of the AdApt transfer vector have been prepared, named AdApt ires-GFP, AdApt HIF-1a-ires-GFP and AdApt EPAS1-ires-GFP. In these vectors, for convenience, AdApt was modified such that inserted genes (i.e. HIF-1a or EPAS1) expressed from the powerful cytomegalovirus (CMV) promoter were linked to the green fluorescent protein (gfp) marker, by virtue of an internal ribosome entry site (ires). Therefore presence of green fluorescence provides a convenient indicator of viral expression of HIF-1a or EPAS1 in transduced mammalian cells. The control vector AdApt ires-GFP was used to allow discrimination between effects of the inserted genes (i.e. HIF-1a or EPAS1) to that of potential non-specific effects of adenoviral transduction or GFP expression. Standard subcloning methods were used to construct the adenoviral constructs as described in detail elsewhere (see co-pending, co-owned International patent application PCT/GB01/00758; Example 2).

The adenoviral transfer vectors AdApt HIF-1a-ires-GFP and AdApt EPAS1-ires-GFP, were verified prior to production of adenoviral particles, for their ability to drive expression of functionally active HIF-1a or EPAS1 protein from the CMV promoter in mammalian cells. This was achieved by transient transfection luciferase-reporter assays as described (Boast K et al *Hum Gene Ther.* 1999 Sep 1;10:2197-208).

Using the aforementioned Introgene adenoviral system, caesium-banded, pure adenoviral particles were produced for each of the vectors AdApt ires-GFP, AdApt HIF-1a-ires-GFP and AdApt EPAS1-ires-GFP. Following the Introgene manual, adenoviral preparations were quantitated by spectrophotometry, yielding values of viral particles (VP) per milliliter.

Primary human macrophages isolated as described above, were washed and resuspended in DMEM (Gibco, Paisley, UK) supplemented with 4% fetal bovine serum (Sigma). $5x10^6$ cells were plated into nine individual 10cm Primeria (Falcon) tissue culture dishes containing medium plus adenovirus as

shown below (experimental design), to give a total volume of 10 ml per plate. Two doses of adenovirus were used; $5.3x10^8$ viral particles / ml (low) and $1.6x10^9$ viral particles / ml (high). These amounts were chosen following a series of titration experiments. Following culture for 16 hr, during which the macrophages adhere to the plate and are infected by the adenoviral particles, the medium was removed and replaced by IMDM medium (Gibco, Paisley, UK) supplemented with 2% human AB serum. A further 24 hr period of culture was allowed prior to experimentation, to allow gene expression from the transduced adenovirus. Gene transduction was verified by visually assessing gfp expression and expression of the viral HIF-1a and EPAS1 genes was determined by real time quantitative RT-PCR using an ABI Prism 7700 TaqMan and CyberGreen protocol. For the high doses of virus, the total levels of HIF-1a or EPAS1 mRNA present in the transduced cells were increased by 10-30 fold.

For experimentation with conditions of hypoxia, identical culture dishes were divided into two separate incubators: One at 37 degrees, 5% CO2, 95% air (=Normoxia; equivalent to 20% Oxygen) and the other at 37 degrees, 5% CO2, 94.9% Nitrogen, 0.1% Oxygen (=Hypoxia). After 6 hours culture under these conditions, the dishes were removed from the incubator, placed on a chilled platform, washed in cold PBS and total RNA was extracted using RNeasy (Qiagen) following the manufacturer's instructions.

Experimental design

Condition	Adenovirus (type)	Adenovirus amount (low=$5.3x10^8$ vp/ml high=$1.6x10^9$ vp/ml)	Oxygen (%)
1	none	none	20
2	AdApt ires-GFP	low	20
3	AdApt ires-GFP	high	20
4	AdApt ires-GFP	low	0.1
5	AdApt ires-GFP	high	0.1
6	AdApt HIF-1a-ires-GFP	low	0.1
7	AdApt HIF-1a-ires-GFP	high	0.1
8	AdApt EPAS1-ires-GFP	low	0.1
9	AdApt EPAS1-ires-GFP	high	0.1

RNA samples from the experimental conditions shown above were each hybridised to individual copies of the Custom gene array and processed as described earlier. To ensure reproducible data, this was repeated so each RNA sample was hybridised to 4 separate arrays. Therefore a total of 36 arrays were used for this experiment. Data analysis was done taking the mean signal of each spot from the four array replicates of each RNA sample. When displayed graphically, standard error of the mean is displayed as

the error bar. Expression values were calculated so that they represent the fold-change ratio as compared to condition#1, i.e. untreated cells.

For genes shown in Table 4 it can be seen that in cells transduced by the control adenovirus AdApt ires-GFP there is a response to hypoxia (conditions 4,5) as compared to in normoxia (conditions 2,3). However this response is significantly greater when the natural hypoxia response is amplified by overexpression of HIF-1alpha from the adenovirus AdApt HIF-1a-ires-GFP (conditions 6,7). Furthermore, this effect is usually dependent on the amount of HIF1alpha overexpression (i.e. greater in condition 7 compared to 6). This same data is displayed graphically in Figure 2. It can be seen that these genes encode metallothionein proteins. One of these (Nucleotide Seq ID No. 84; Protein Seq ID No. 83) is a novel member of the matallothionein family. Several metallothionein genes are known in the art to be activated by hypoxia, supporting the usefulness of this data.

For genes shown in Table 5 and Figure 3 it can be seen that in cells transduced by the control adenovirus AdApt ires-GFP there is a response to hypoxia (conditions 4,5) as compared to in normoxia (conditions 2,3). However this response is significantly greater when the natural hypoxia response is amplified by overexpression of EPAS1 from the adenovirus AdApt EPAS1-ires-GFP (conditions 8,9).

In the case of the protein encoded by Seq ID No. 24, results are available independently for two separate cDNA clones representing non-overlapping regions of the same full length gene.

In the case of the protein encoded by Seq ID No. 86 (EGL nine (C.elegans) homolog 3), additional evidence is described above in support of the function of this protein. Furthermore, real time quantitative RT-PCR analysis of this gene using an ABI Prism 7700 TaqMan and CyberGreen protocol, has been performed, to verify and more accurately quantitate the upregulation of EGL nine (C.elegans) homolog 3 in response to hypoxia and EPAS1 adenoviral overexpression. The main difference between the array-based and real time quantitative RT-PCR methodologies is that the latter is far more sensitive and therefore can detect expression in the off-state (here normoxia) for weakly expressed genes. This data has shown an induction ratio of 819-fold for EGL nine (C.elegans) homolog 3 in response to hypoxia with additional EPAS1 expression, from RNA generated from an independent experiment. This data was normalised to beta actin.

Similarly another weakly-expressed EPAS1-induced gene, Semaphorin 4b (Seq ID No. 91/92; see additional discussion above) has been determined using real time quantitative RT-PCR methodology, showing an actin-normalised induction ratio of 30.1 is found (data not shown).

For the gene shown in Table 6 and Figure 4 it can be seen that in cells transduced by the control adenovirus AdApt ires-GFP, there is a negative response to hypoxia (conditions 4,5) as compared to in normoxia (conditions 2,3). However, this response is significantly greater when the natural hypoxia response is amplified by overexpression of HIF1 alpha or EPAS1 (conditions 6,7,8,9).

Example 2: Differences in the hypoxia responses of resting and activated macrophages.

Macrophages accumulate at hypoxic areas in various disease states, including cancer, rheumatoid arthritis, atherosclerosis and wound healing. At these sites macrophages activation is liable to occur, such as in response to T-cell derived gamma interferon. For instance, in atherosclerotic plaques there is an accumulation of both T-cells and macrophages, and these are known to interact with one another (reviewed in Lusis AJ, Atherosclerosis. Nature. 2000 Sep 14;407(6801):233-41).

It is well established that the macrophage has a significant role in the pathology of the above diseases involving hypoxia, and that most functions of the macrophage (including inflammatory functions) are greatly increased following activation. Therefore any therapeutic strategy aimed at the hypoxic macrophage, needs to also consider the effects of macrophage activation and possible cross talk between the responses to macrophage activation and hypoxia.

2.1: Research Genetics Human GeneFilters

This work was carried out using Research Genetics Human GeneFilters, which contain DNA derived from clones of the IMAGE cDNA collection, representing genes of varying degrees of characterisation. A series of 6 arrays of human genes were used (GeneFilters GF200-205), potentially covering a total of 31,104 genes. Generally, single genes are represented only once in these arrays. However, sometimes IMAGE clones initially thought to represent separate genes, upon re-analysis were found to be different regions of the same gene. Here we have presented data for all clones individually, though they possess the same UniGene ID and gene name. An example is Hypothetical protein FLJ20037.

The methodology for Research Genetics arrays is similar in principle to that described for the array screening of clones from subtracted libraries. There are several attributes to this method: Relatively small amounts of RNA can be labelled to make cDNA probes, in a single step reaction, and probes are labelled with the same chemical group (33P), so there are no errors introduced as a result of using different dyes, which may differ in stability etc. Using a Phosphorimager allows detection over a wide range of intensities (over 4 logs). Overall it is interesting to note a recent study, which has favourably re-evaluated the performance of the nylon based array, as compared with the glass-based microarray method (Bertucci F *et al*, *Hum Mol Genet* 8:1715-1722 (1999)).

Experiments were done essentially as described in the Research Genetics GeneFilters protocol. Duplicate copies of each array from the same production batch, were used and hybridised in parallel with labelled RNA isolated from normoxic and hypoxic primary human macrophages. Hybridised arrays were scanned twice using a Molecular Dynamics Storm phosphorimager, and both images were analysed to ensure reproducibility. Furthermore, the experiments were repeated using the same RNA samples, but with different array lot numbers, again to ensure reproducibility.

Analysis was performed using Research Genetics Pathways software, with normalisation using the 'all data points' option. Analyses were output as spreadsheets and filtered to remove data points where the signal intensity was less than 4-fold above the general background for the experimental condition with the higher signal (hypoxia or normoxia depending on whether hypoxia causes induction or repression). Sometimes expression in the lower state was not significantly above background, and the ratio will therefore be underestimated. Ratios were calculated by normalised signal intensity in hypoxia divided by normoxia. Changes were verified visually from the original array images.

In this manner, comparisons were made between normoxia and hypoxia in resting macrophages. The whole procedure was then repeated for activated macrophages, to investigate possible differences in the response to hypoxia. It is possible that potential differences for certain genes could be correlated with changes in expression resulting from activation, prior to challenge with hypoxia. To explore this possibility, comparisons were made between resting and activated macrophages, both in normoxia. Since some of the genes we have identified as being activated by hypoxia have very low hybridisation signals in normoxia (for both resting and activated macrophages), this comparison was not possible.

We have found various patterns of gene expression changes occurring in response to hypoxia, related to the activation state of macrophages, which are presented below. Such a range of responses, specific to various subsets of genes, was not expected, and contradicts a view that the hypoxia response is a largely a generic mechanism.

Table 7 shows genes that are induced by hypoxia to a similar degree in resting and activated macrophages.

Table 8 shows genes that are induced by hypoxia to a greater degree in resting macrophages, as compared to activated macrophages. These data are presented illustratively in Figure 5.

Data from Table 8/Figure 5 reveals several unexpected observations.

A) From the final column it can be seen that macrophage activation in the absence of hypoxia, causes induction of many of these genes. This suggests that the signalling pathways resulting from activation and hypoxia might converge to a single transcriptional regulator, causing macrophage activation to pre-empt the response to subsequent hypoxia. This is exemplified most strikingly for

Interleukin 8, which is dramatically induced in response to macrophage activation, but shows no additional response to hypoxia.

B) Genes in rows 11, 13 and 14 have no response to hypoxia following macrophage activation, though there is not a preceding large increase in expression in response to macrophage activation alone. This suggests that in the activated macrophage, the necessary signalling pathway or transcriptional regulator is not functional.

C) Although Table 8 was produced electronically, without selecting genes based on their names, it can be seen that genes encoding proteins of the metallothionein family feature strongly.

Table 9 shows genes which are induced by hypoxia to a greater degree in activated macrophages, compared to resting macrophages. These data are presented illustratively in Figure 6.

In Table 7, there are several genes for which hypoxia/ normoxia ratios were only obtained for activated macrophages, such as Cox-2 (see row 47). For these genes, macrophage activation usually increases expression of the gene to detectable levels, thus allowing the study of subsequent changes in response to hypoxia. It is likely that these genes are not significantly expressed in resting macrophages irrespective of hypoxia, and therefore the hypoxia response is probably specific to activated macrophages.

Certain genes respond to hypoxia by decreasing mRNA expression (repression), and these genes therefore have hypoxia/normoxia ratios of < 1.0. This phenomenon is known in the field of hypoxia, although the mechanism is obscure. Data is presented in tables 7-9, which unexpectedly shows that this hypoxia-induced repression for specific genes is not a generic process, but is dependent on the cellular context. In Table 10/ Figure 7, genes are presented that are hypoxia-repressed to a greater degree in activated (column 7) compared with resting (column 8) macrophages. Prior to any hypoxic challenge, these gene are induced to varying degrees, in response to macrophage activation (column 9), suggesting a shared mechanism for these separate responses. From Table 10, genes in rows 1-6 show that macrophage activation is necessary to obtain any response to hypoxia. In resting macrophages, these genes are not responsive to hypoxia at all.

Strikingly, Table 10/ Figure 7 shows that seven separate genes encoding chemokine proteins (Monocyte chemotactic protein 1, Macrophage inflammatory protein 1b, Monocyte chemotactic protein 3 and Small inducible cytokine A3, Monocyte chemotactic protein 2, Macrophage inflammatory protein 2a and Macrophage inflammatory protein 2 precursor) are more strongly repressed in activated macrophages as compared to resting macrophages. These genes are also among the most inducible in response to activation alone, in normoxia (column 9). These findings are of potential utility in view of the great significance of chemokines to inflammatory disease. For example, macrophage chemotactic factor 1

(Table 10, row 19) is key to the pathological role of the macrophage in atherosclerosis ("Chemokines and atherosclerosis" Reape TJ and Groot PHE, Atherosclerosis 147: 213-225, 1999).

Genes in rows 20-30 of Table 10, were not detectably expressed in resting macrophages, irrespective of hypoxia. Table 11 shows other genes that were down-regulated in response to hypoxia in macrophages.

Example 3: Tissue-specific hypoxia regulation of gene expression by an analysis of a series of primary human cell cultures.

Equivalent cultures of non-immortalised, non-transformed primary human cells of 10 distinct types, were cultured in either normoxia or were exposed to hypoxia for 6 hr and 18 hr, and gene expression changes were determined. To the inventors' knowledge, this is the first time that such a study has been reported. Moreover, unlike the vast majority of information in the public domain relating to genes responsive to hypoxia, all of these cells were human and were cultured without any modifications following isolation from the human donors. By using primary cells rather than cell lines or immortalised cultures, the findings of this work more accurately represents the situation in the human body.

Most cell types were obtained from Clonetics (distributed by BioWhittaker, Walkersville, MD) and cultured according to the manufacturer's recommendations, unless where otherwise shown. #1:adipocyte (Clonetics CC-2568; derived from subcutaneous adult adipose tissue), #2:cardiomyocyte (Clonetics CC-2582; derived from fetal tissue; prior to experimentation cultured in minimal medium: DMEM, 4% Horse serum), #3:endothelial (TCS CellWorks ZHC-2101 human umbilical vein endothelial cells), #4:fibroblast (Clonetics CC-2511 dermal fibroblasts derived from adult tissue), #5:hepatocyte (Clonetics CC-2591, derived from adult tissue), #6:macrophage (derived from human blood as described elsewhere in the specification), #7:mammary epithelial (Clonetics CC-2551; derived from adult tissue), #8:monocyte (derived from human blood as described elsewhere in the specification but without the 7 day differentiation culture period), #9:neuroblastoma (neuroblastoma-derived cell line SH-SY5Y), #10:renal epithelial (Clonetics CC-2556; derived from fetal tissue), #11:skeletal muscle myocyte (Clonetics CC-2561; derived from adult tissue). A non-primary cell type (#9) was used to represent neurons, since primary human neurons are difficult to source. Therefore a total of 11 cell types are compared. It should be noted that RNA from hepatocytes at the 16hr timepoint of hypoxia was not available for this work.

Genes which were induced or repressed preferentially in particular cell type(s) were identified by hybridisation of the RNA samples to the custom gene array, as described in Examples 1b and 1c. Each RNA sample was hybridised to duplicate or triplicate arrays, to ensure reproducible data, and was analysed using GeneSpring software. Data from replicate arrays were merged during analysis to generate mean values. Data normalisation was achieved per-array using the aforementioned list of control genes, such that differences in RNA labelling or hybridisation due to experimental variation were corrected by

referencing each gene to the mean value of the reference genes on the same array. Also, for each gene, expression values were obtained which represent the value in each experimental condition (e.g. macrophages 6hr hypoxia) as compared to the median of value of that gene throughout the full range of experimental conditions (i.e. from all cell types). This transformation does not alter the relative values of any gene between the different experimental conditions, and is done since these is no obvious single reference experimental condition to create ratio values. This is common in microarray data analysis.

Table 12 shows the full dataset of this analysis. From this it can be seen that certain genes respond to hypoxia differently, depending on the particular cell type. This information is valuable in identifying biological targets for the development of therapeutic and diagnostic products. Not only does it indicate a particularly significant role for these genes in the specific cell type implicated in a disease, but it also identifies that any therapeutic product is less likely to produce problematic toxicological effects. Data shown in Table 12 and the derived figures, are reproducible, and are an accurate determination of mRNA expression levels. This may be confirmed by independent means, such as quantitative real time RT-PCR.

Certain genes from Table 12 will be presented for illustration.

Genes with a greater response in monocytes or macrophages

Since monocytes and macrophages are similar cell types, the latter derived from the former, they will be analysed together.

Expression profiles of 11 genes showing hypoxia-induced changes in gene expression which are most pronounced in monocytes or macrophages are shown in Figures 8-18. These genes correspond to:

Seq ID:339/340 CYP1 (cytochrome P450, subfamily XXVIIB)
Seq ID:357/358 interleukin 1 receptor antagonist
Seq ID:375/376 Regulator of G-protein signalling 1
Seq ID:389/390 GM2 ganglioside activator protein
Seq ID:405/406 Alpha-2-macroglobulin
Seq ID:475/476 Ecotropic viral integration site 2A=
Seq ID:433/434 high affinity immunoglobulin epsilon receptor beta (CFFM4)
Seq ID:431/432 Pleckstrin
Seq ID:469/470 cytokine effector of inflammatory response SCYA3L
Seq ID:79/80 Novel PI-3-kinase adapter
Seq ID:21/22 Hypothetical protein PRO0823

It will be appreciated that the majority of these genes have a known biological function in immunity/ inflammation, consistent with the known function of the monocyte/ macrophage. Further to this knowledge, this data identifies that in hypoxic disease sites where monocyte/ macrophages make up a

significant proportion of the cell types, such as in rheumatoid arthritis synovial membranes, that these genes are possible therapeutic targets.

Ecotropic viral integration site 2A (Seq ID:475/476)

For example, the gene illustrated in Figure 8, Ecotropic viral integration site 2A (Seq ID:475/476) is induced in hypoxic monocytes to a level over 25 times higher than the median expression level of this gene throughout the other cell types. This gene, of unknown function, is located on Chromosome 17q11.2 close to genes with immune functions. Presented elsewhere in this specification is data showing that expression of Ecotropic viral integration site 2A is downregulated in response to the inflammatory cytokine interferon gamma. These novel data provide evidence that Ecotropic viral integration site 2A is a novel target for inflammatory conditions involving hypoxia and monocytes.

Novel PI-3-kinase adapter Seq ID:79/80 Clone p1E9 (EST accession R62339).

Another example, in Figure 9a, is Seq ID:79/80 (EST accession R62339). It is seen that in hypoxic macrophages, this gene is expressed at 6-fold higher levels than the median expression level of this gene throughout the other cell types. Therefore, the levels of the encoded protein in hypoxic monocytes/ macrophages, as found at various disease sites, are likely to be higher than in other cell types not involved in the disease process or present at the site of disease. This illuminates a novel utility of this gene as a target for the development of therapeutic products for diseases involving monocytes/ macrophages and hypoxia.

The data that led to the generation of this Figure are as follows:

Cell type	*Oxygen*	*Normalised expression* (clone p1E9 / SeqID:79/80)
adipocyte	normoxia	1.54
adipocyte	hypoxia 6hr	0.89
adipocyte	hypoxia 18hr	1.48
cardiomyocyte	normoxia	1.18
cardiomyocyte	hypoxia 6hr	1.80
cardiomyocyte	hypoxia 18hr	1.53
endothelial	normoxia	0.68
endothelial	hypoxia 6hr	0.82
endothelial	hypoxia 18hr	0.60
fibroblast	normoxia	0.60
fibroblast	hypoxia 6hr	0.64
fibroblast	hypoxia 18hr	0.73
hepatocyte	normoxia	0.92
hepatocyte	hypoxia 6hr	1.62
macrophage	normoxia	4.20
macrophage	hypoxia 6hr	3.97
macrophage	hypoxia 18hr	6.19
mammary epithelial	normoxia	0.25
mammary epithelial	hypoxia 6hr	0.42

mammary epithelial	hypoxia 18hr	0.18
monocyte	normoxia	2.33
monocyte	hypoxia 6hr	3.63
monocyte	hypoxia 18hr	5.01
neuroblastoma	normoxia	0.93
neuroblastoma	hypoxia 6hr	0.80
neuroblastoma	hypoxia 18hr	0.85
renal epithelial	normoxia	0.57
renal epithelial	hypoxia 6hr	0.61
renal epithelial	hypoxia 18hr	0.61
skeletal myocyte	normoxia	1.58
skeletal myocyte	hypoxia 6hr	1.37
skeletal myocyte	hypoxia 18hr	1.17

To substantiate the array-based data, the same RNA samples were examined by real time quantitative RT-PCR. The advantages of this method are that it is more sensitive and because two gene-specific primers are used, the data will be more specific to the gene in question.

RNA from the above samples (except for the hepatocyte RNA which was unavailable) was Dnase I-treated prior to reverse transcription to remove possible contaminating genomic DNA and was reverse transcribed using an oligo $dT_{(15)}$ primer and Superscript II reverse transcriptase. These samples were used as template for PCR reactions using primers specific to EST accession R62339 or to beta-actin. Primer sequences were as follows:

Novel PI-3-kinase adapter Seq ID:79/80 Clone p1E9 (EST accession R62339).

Forward Primer 5' GCC CTT AGT TTT TCA CTT CTT CGT 3'

Reverse Primer 5' CCT TAA GAT CCA TTC TCA TTG CTG AT 3'

Beta Actin

Forward Primer 5' GCC CTG AGG CAC TCT TCC A 3

Reverse Primer 5' GCG GAT GTC CAC GTC ACA 3'

All RT-PCR reactions were performed using an ABI Prism 7700 Sequence Detector system. For each Q-PCR run, a master mix was prepared with 2x SYBR Green I master mix (Applied Biosystems) and primers at 5μM. Two microlitres of respective diluted cDNA were added to PCR master mixture, amounting to 25μL. The thermal cycling conditions comprised 50°C for 2 minutes, 95°C for 10 minutes, 40 cycles at 95°C for 15 seconds, and 60°C for 1 minute. PCR reactions were set up in 96 well format with duplicate amplifications for each data point including 8 serial cDNA dilutions (0.2, 0.1, 0.05, 0.025, 0.01, 0.005, 0.001 and 0.0001) of macrophage treated with 18 hours hypoxia to compose a standard curve, a no template control, no amplification control lacking reverse transcriptase, and each cDNA sample at a dilution value of 0.1. The experiment for the novel PI3K adapter was carried out in triplicate for reproducibility which were later determined by linear regression analysis. Data was analysed with

necessary adjustment of the default baseline and threshold line using ABI Prism 7700 software. The C_t value, an important raw data for each sample, was calculated as the cycle number at which the ΔRn crosses the baseline. For each run, a standard curve was constructed by plotting a graph with mean C_t values from 8 data points from standard sample against log input of the corresponding dilution values with a best fit trend line. From the trend line, the formula 'y=mx+c' was created according to the y-intercept and slope of standard curve which then were used for calculating the log input amount of the experimental cDNA samples, as related to the calibration sample. Data for the Novel PI-3-kinase adapter was normalized to that of beta-actin to correct for potential differences in efficiency of cDNA synthesis between the RNA samples.

From the TaqMan data the specificity to monocytes and macrophage found from the array data is confirmed and found to be even more pronounced (see Figure 9b). The data presented in the Figure are listed below. In the data listed below, the normalized expression values are multiplied by 1000 for clarity.

Cell type	Oxygen	Normalised expression (clone p1E9 / SeqID:79/80)
adipocyte	normoxia	0.050
adipocyte	hypoxia 6hr	0.007
adipocyte	hypoxia 18hr	0.015
cardiomyocyte	normoxia	0.163
cardiomyocyte	hypoxia 6hr	0.037
cardiomyocyte	hypoxia 18hr	0.222
endothelial	normoxia	3.093
endothelial	hypoxia 6hr	0.059
fibroblast	normoxia	0.527
fibroblast	hypoxia 6hr	0.043
fibroblast	hypoxia 18hr	0.037
macrophage	normoxia	404.593
macrophage	hypoxia 6hr	503.026
macrophage	hypoxia 18hr	1162.056
mammary epithelial	normoxia	0.026
mammary epithelial	hypoxia 6hr	0.068
mammary epithelial	hypoxia 18hr	0.112
monocyte	normoxia	565.471
monocyte	hypoxia 6hr	657.465
monocyte	hypoxia 18hr	979.048
neuroblastoma	normoxia	8.482
neuroblastoma	hypoxia 6hr	7.104
neuroblastoma	hypoxia 18hr	4.707
renal epithelial	normoxia	17.898
renal epithelial	hypoxia 6hr	9.831
renal epithelial	hypoxia 18hr	10.929
skeletal myocyte	normoxia	0.930
skeletal myocyte	hypoxia 6hr	0.638
skeletal myocyte	hypoxia 18hr	1.627

There are several technical reasons why the results from the array-based data might be more pronounced in the Taqman results - the lower sensitivity of the array-based method means that genes which are not expressed will be detected as a background signal. Also the array method is more likely to suffer from cross-hybridisation between similar genes.

The TaqMan data illustrates dramatically the concept that the hypoxia response is not just a generic response found in all cell types, relating to generic cell processes such as metabolism.

Database searches for gene sequences showing identity with IMAGE clone acc:R62339 reveal that there are no matching human sequences of any type other than ESTs. This includes full length cDNAs, truncated cDNAs, gene sequences from chromosomal data or hypothetical protein gene sequences. Therefore the human gene represented by IMAGE clone acc:R62339 is a novel human gene.

Although this human EST is unannotated, by comparison with mouse sequence data (acc AF293806), it appears likely to encode a novel human Phosphoinositol 3-kinase (PI3-kinase) adapter molecule, homologous to the recently described mouse gene, BCAP. This class of molecule, involved in intracellular signalling, have been shown to have utility as a drug target (see Stein RC *et al*, "PI3-kinase inhibition: a target for drug development" *Mol Med Today*. 2000 Sep;6(9):347-57). PI3-kinases are key to many cellular processes relevant to human disease, including proliferation, apoptosis and inflammation. The data presented for the gene encoded by Seq ID:79/80 provides evidence that the encoded protein is a novel drug target in humans, specifically targeting monocyte/ macrophages at hypoxic disease sites.

In the publication relating to murine BCAP, the protein is identified as an adapter molecule connecting the non-receptor protein tyrosine kinase Syk to the p85 subunit of PI3-kinase, and therefore to the pivotal signalling pathways centred around PI3-kinase (Okada T *et al* "BCAP: the tyrosine kinase substrate that connects B cell receptor to phosphoinositide 3-kinase activation." *Immunity*. 2000 13:817-27). Although, in this report, Syk is acting as the intracellular signalling component of the B cell antigen receptor, which is present exclusively on B-cells, Syk has been shown to initiate intracellular signalling from other cell surface receptors which are expressed on macrophages, including the Fc gamma receptor, the chemokine receptor CCR5 and macrophage-expressed CD8 (Darby C et al "Stimulation of macrophage Fc gamma RIIIA activates the receptor-associated protein tyrosine kinase Syk and induces phosphorylation of multiple proteins including p95Vav and p62/GAP-associated protein". *J Immunol*. 1994 152:5429-37) (Kedzierska K et al "FcgammaR-mediated phagocytosis by human macrophages involves Hck, Syk, and Pyk2 and is augmented by GM-CSF." *J Leukoc Biol*. 2001 Aug;70(2):322-8.), (Ganju RK et al "Beta-chemokine receptor CCR5 signals through SHP1, SHP2, and Syk." *J Biol Chem*. 2000 275:17263-8.), (Lin TJ et al "Activation of macrophage CD8: pharmacological studies of TNF and IL-1 beta production." *J Immunol*. 2000 164:1783-92.).

Indeed, syk has been validated as target in macrophages to inhibit inflammatory activities of this cell type (Stenton GR et al "Aerosolized Syk antisense suppresses Syk expression, mediator release from macrophages, and pulmonary inflammation." *J Immunol.* 2000 Apr 1;164(7):3790-7.).

Additional to the finding that the probable human orthologue of the adapter molecule BCAP is preferentially hypoxia-induced in human monocytes/ macrophages, we also find from data generated by the custom array, that the protein acting immediately upstream of BCAP (i.e. Syk) is also regulated by hypoxia in this novel cell type specific manner, greatly increasing the biological significance of the original finding (see Figure 9c). The data used to generate this Figure are presented below for clarity.

Cell type	Oxygen	Normalised expression (of syk)
adipocyte	normoxia	2.6573591
adipocyte	hypoxia 6hr	1.499927
adipocyte	hypoxia 18hr	1.1115488
cardiomyocyte	normoxia	0.8357341
cardiomyocyte	hypoxia 6hr	2.161058
cardiomyocyte	hypoxia 18hr	0.90880114
endothelial	normoxia	0.60265505
endothelial	hypoxia 6hr	0.56874704
endothelial	hypoxia 18hr	0.43321633
fibroblast	normoxia	0.8542026
fibroblast	hypoxia 6hr	0.7657573
fibroblast	hypoxia 18hr	0.784982
hepatocyte	normoxia	0.5238476
hepatocyte	hypoxia 6hr	0.8465495
macrophage	normoxia	4.272981
macrophage	hypoxia 6hr	6.144931
macrophage	hypoxia 18hr	10.278416
mammary epithelial	normoxia	1.1023632
mammary epithelial	hypoxia 6hr	2.7382789
mammary epithelial	hypoxia 18hr	0.7985004
monocyte	normoxia	6.052118
monocyte	hypoxia 6hr	8.6809225
monocyte	hypoxia 18hr	11.58468
neuroblastoma	normoxia	1.0230793
neuroblastoma	hypoxia 6hr	1.089154
neuroblastoma	hypoxia 18hr	0.7689335
renal epithelial	normoxia	0.88565326
renal epithelial	hypoxia 6hr	1.2609364
renal epithelial	hypoxia 18hr	0.6242461
skeletal myocyte	normoxia	1.3959162
skeletal myocyte	hypoxia 6hr	0.91255134
skeletal myocyte	hypoxia 18hr	0.64795935

In summary, we have shown here that a novel human gene encoding a predicted signalling protein relevant to human disease is activated by hypoxia, specifically in monocytes and macrophages. This data

is validated by non-array based means. Furthermore, we identify the protein immediately upstream of this signalling system as being co-regulated in this manner too. Therefore the human PI3-kinase adapter encoded by IMAGE clone acc: R62339 and the non-receptor tyrosine kinase Syk are both identified here for the first time as therapeutic targets for diseases involving hypoxic macrophages, including Rheumatoid arthritis, chronic occlusive pulmonary disease, atherosclerosis and cancer. Because both genes are preferentially expressed in hypoxic macrophages, toxicity effects of therapeutic products directed at the encoded proteins are likely to be limited.

As discussed in detail above, fragments and functional equivalents of the PI-3-kinase adapter protein represented in Seq ID:79/80 and other equivalent proteins are included within the present invention, in addition to ligands that bind specifically to these proteins. Furthermore, the invention also embraces purified and isolated nucleic acid molecules encoding these proteins, fragments and functional equivalents, vectors containing such nucleic acid molecules and host cells transformed with these vectors.

Regulator of G-protein signalling 1 (Seq ID:375/376)

Another intracellular signalling protein, Regulator of G-protein signalling 1 (RGS1; Seq ID:375/376), in shown in Figure 10. Here the expression levels in the hypoxic monocyte is 30-fold higher than the median expression level of this gene throughout the other cell types. The function of this protein is to negatively regulate G protein signalling pathways, and inhibit chemokine-induced cell migration of immune cells (Moratz C et al *J Immunol*, 2000 164:1829-38 and Denecke B et al *J Biol Chem*. 1999 274:26860-8.).

Our data suggests that this gene is preferentially expressed in macrophages, consistent with the findings of Denecke B et al (*J Biol Chem*. 1999 274:26860-8.). Our novel finding that expression is even further enhanced by hypoxia illuminates a mechanism by which cell migration is inhibited in hypoxia, leading to an accumulation of these cells at pathological sites of hypoxia. This mechanism is novel and distinct to other mechanisms proposed in the art to explain this key aspect of hypoxia and inflammation (for example: Grimshaw MJ et al "Inhibition of monocyte and macrophage chemotaxis by hypoxia and inflammation--a potential mechanism." *Eur J Immunol.* 2001 31:480-9).

Furthermore, Figure 10 shows that Regulator of G-protein signalling 1 is upregulated during differentiation of monocytes to macrophages, with significance to changes in cell motility. This discovery therefore provides that inhibitors of RGS1 have utility in increasing the motility of macrophages that are used for cell-based therapies. Accordingly, one embodiment of this aspect of the invention provides for the use of an inhibitor of RGS1 in therapy, by increasing the motility of macrophage cells.

GM2 ganglioside activator protein

The gene shown in Figure 11, GM2 ganglioside activator protein, was originally characterised as a lysosomal co-factor required for degradation of gangliosides. It has been proposed to have alternative

roles as a secreted protein, and can bind and inhibit the actions of the inflammatory mediator, platelet activating factor (Rigat B et al *Biochem Biophys Res Commun.* 1999 258:256-9.).

Our novel finding, presented in Figure 11, shows that GM2 ganglioside activator protein is induced by hypoxia, preferentially in macrophages, suggesting an influence on the inflammatory functions of the macrophage in hypoxia.

In Figures 15-18, genes are shown which are expressed preferentially in the monocyte/ macrophage, but which are *decreased* in expression in response to hypoxia. Being expressed at highest levels in the monocyte/ macrophage, these genes are more likely to be significant to the biological functions of this cell type.

Interleukin 1 receptor antagonist (Seq ID:357/358)

In Figure 15, the gene interleukin 1 receptor antagonist (Seq ID:357/358) is seen to be down-regulated by hypoxia in the macrophage. Since the function of the encoded protein is anti-inflammatory, then down-regulation of this gene would be expected to have a pro-inflammatory effect. Therefore, corrective expression of the gene, would be expected to produce therapeutic effects in inflammatory disorders involving macrophages and hypoxia, such as Rheumatoid Arthritis (Hollander AP *et al. Arthritis Rheum.* 2001 44:1540-4). This correlates with effects seen from the application the drug Anakrina / Kineret™ developed by Amgen. This supports the applicability of the genes disclosed herein as novel targets for therapeutic products.

The example of gene interleukin 1 receptor antagonist also provides good exemplification of the concept that different cell types respond to hypoxia differently. Here, not only are there quantitative differences, but also qualitative differences in that this gene is *down*-regulated by hypoxia in macrophages, but *up*-regulated by hypoxia in several other cell types, such as renal epithelial cells (see Figure 15). Such findings are not documented in the art.

The dataset of Table 12 also contains genes which are induced preferentially in monocyte/ macrophages and also in some but not all other cell types tested. Several of these genes are present as multiple clones on the gene array, giving separate data, therefore adding extra confidence to the conclusions. These genes, presented in Figures 19-28 correspond to:

SeqID:313/314 adipophilin
SeqID:163/164 Hypothetical protein FLJ13511
SeqID:267/268 Osteopontin
SeqID:17/18 Hematopoietic Zinc finger protein
SeqID:137/138 CYP1B1
SeqID:325/326 CYP1B1

It will also be seen that in the case of CYP1B1 (clones p1F16 and p1E3) the hypoxia response in monocytes / macrophages is qualitatively different to the other cell types tested, in that the gene is up-regulated rather than down-regulated in response to hypoxia.

Genes with a greater response in endothelial cells

The dataset of Table 12 also contains genes which are induced preferentially in endothelial cells, a cell type key to the process of angiogenesis, in response to hypoxia. These genes are as follows, and are presented in Figures 29-31:

SeqID:205/206 Hypothetical protein FLJ22690
SeqID:65/66 cDNA DKFZp586E1624
SeqID:197/198 EST

Genes with a greater response in hepatocytes

The dataset of Table 12 also contains genes which are induced preferentially in hepatocytes, in response to hypoxia. These genes are presented in Figures 32a and 33-38. It is noted that most of these genes, including hqp0376, encode proteins of the metallotheionein family. Furthermore, close inspection of these data reveals that the fold induction in hypoxia compared to normoxia for monocyte/ macrophages are very high, though the absolute levels of expression are below that of hepatocytes.

SeqID:85/86 EGL nine (C.elegans) homolog 3
SeqID:83/84 Novel Metallothionein
SeqID:337/338 Hypothetical protein hqp0376 (a metallotheionein)
SeqID:265/266 Metallothionein 2A
SeqID:243/244 Metallothionein 1G
SeqID:141/142 Hepcidin antimicrobial peptide
SeqID:239/240 Metallothionein 1H

EGL nine (C.elegans) homolog 3

As described above, it has been discovered that a polypeptide encoded by a gene identified from the EST recited in SEQ ID No 86, having the Protein accession number BAB15101 (encoded by Homo sapiens cDNA: FLJ21620 fis, clone COL07838 Nucleotide accession AK025273) is regulated by hypoxia. Other public domain sequences corresponding to this gene include Homo sapiens cDNA: FLJ23265 fis, clone COL06456 Nucleotide accession AK026918. Accordingly, when referring in the present specification to the EST recited in SEQ ID No 86, it is intended that these gene and protein sequences are also embraced. This gene was identified using Research Genetics Human GeneFilters arrays, which contain an EST

corresponding to the gene (accession number R00332). The gene is now termed EGL nine (C.elegans) homolog 3.

There are no reports that describe the function of this human gene. However, a high degree of amino acid homology is observed between the protein encoded by this gene, and a rat protein called "Growth factor responsive smooth muscle protein" or "SM20" (Nucleotide accession U06713; Protein accession A53770). An alignment of single letter amino acid sequences is shown below. Over the highlighted region there is 97% amino acid similarity and 96% amino acid identity.

```
A53770     (1) MTLRSRRGFLSFLPGLRPPRRWLRISKRGPPTSHWASPALGGRTLHYSCR
BAB15101   (1) ----------------------------------------------------
                51                                                100
A53770    (51) SQSGTPFSSEFQATFPAFAAKVARGPWLPQVVEPPARLSASPLCVRSGQA
BAB15101   (1) ----------------------------------------------------
                101                                               150
A53770   (101) LGACTLGVPRLGSVSEMPLGHIMRLDLEKIALEYIVPCLHEVGFCYLDNF
BAB15101   (1) ----------------MPLGHIMRLDLEKIALEYIVPCLHEVGFCYLDNF
                151                                               200
A53770   (151) LGEVVGDCVLERVKQLHYNGALRDGQLAGPRAGVSKRHLRGDQITWIGGN
BAB15101  (35) LGEVVGDCVLERVKQLHCTGALRDGQLAGPRAGVSKRHLRGDQITWIGGN
                201                                               250
A53770   (201) EEGCEAINFLLSLIDRLVLYCGSRLGKYYVKERSKAMVACYPGNGTGYVR
BAB15101  (85) EEGCEAISFLLSLIDRLVLYCGSRLGKYYVKERSKAMVACYPGNGTGYVR
                251                                               300
A53770   (251) HVDNPNGDGRCITCIYYLNKNWDAKLHGGVLRIFPEGKSFVADVEPIFDR
BAB15101 (135) HVDNPNGDGRCITCIYYLNKNWDAKLHGGILRIFPEGKSFIADVEPIFDR
                301                                               350
A53770   (301) LLFSWSDRRNPHEVQPSYATRYAMTVWYFDAEERAEAKKKFRNLTRKTES
BAB15101 (185) LLFFWSDRRNPHEVQPSYATRYAMTVWYFDAEERAEAKKKFRNLTRKTES
                351
A53770   (351) ALAKD
BAB15101 (235) ALTED
```

The high degree of amino acid similarity suggests that the human protein BAB15101 has an equivalent biochemical function to the rat protein A53770 ("Growth factor responsive smooth muscle protein" or "SM20"). Recent publications have shown that SM20 functions to promote apoptosis in neurons (Lipscomb *et al., J Neurochem* 1999; **73**(1):429-32; Lipscomb *et al., J Biol Chem* 2000 Nov 1; [epub ahead of print]). Significantly, SM20 has been shown to be expressed at high levels in the heart (Wax *et al., J Biol Chem* 1994; **269**(17): 13041-7).

It has also been discovered that a polypeptide encoded by a gene identified from the EST recited in SEQ ID No 90, having the Protein accession number CAB81622, is regulated by hypoxia. The encoding human gene has been annotated in the UniGene database as "Similar to rat smooth muscle protein SM-

20"; the nucleotide sequence is contained within the nucleotide accession AL117352. More recently, a longer fragment of this gene has been cloned, named c1orf12, or EGLN1 (Nucleotide accession AAG34568; Protein accession AAG34568). Accordingly, when referring in the present specification to the EST recited in SEQ ID No 90, it is intended that these gene and protein sequences are also embraced.

This distinct human gene, encoding a protein related to SM20 and EGLN3 (BAB15101), is also induced in response to hypoxia. This gene was identified using Research Genetics Human GeneFilters arrays, which contain an EST corresponding to the gene (accession number H56028).

Independently to this, a fragment of this gene has been cloned from a cDNA library derived from hypoxic human cardiomyoblasts, and it has been shown that the gene is increased in expression in response to hypoxia in this cell type (see Table 1 herein; penultimate row). The nucleotide sequence of this cDNA fragment is referred to herein as SEQ ID No 90a.

In the light of this novel discovery reported herein that these human equivalents of SM20 are induced by hypoxia, it is herein proposed that in cardiac ischaemia, the resulting apoptosis is due at least in part, to increased expression of these genes. The therapeutic modulation of the activity of EGLN3 (BAB15101), c1orf12 (AAG34568), CAB81622, SM20 and other equivalent proteins and encoding genes therefore provides a novel means for the treatment of myocardial ischaemia, through the alteration of the propensity of myocardial cells to undergo apoptosis. For example, a suitable treatment may involve altering the susceptibility of ischaemic myocardial tissue to subsequent reperfusion and re-oxygenation, or may involve modulating the susceptibility of chronic ischaemic myocardial tissue (including forms of angina) to later more severe ischaemia, which would result in myocardial infarction. It is submitted that, by way of analogy, cerebral ischaemia may be treated using the same principle.

Although the Applicant does not wish to be bound by this theory, the downstream effects of SM20 and related genes such as EGLN3 (BAB15101), c1orf12 (AAG34568), and CAB81622, namely, apoptosis and angiogenesis might be explained as follows. The apoptotic effect of NGF withdrawal may be mediated by induction of the hypoxia pathway, but may be an aspect of the supposed involvement of the HIF protein in the stress response. HIF1α is induced by reactive oxygen species (see Richard et al. J Biol Chem 2000 Sep 1;275(35):26765-71). This could, in turn, be mediated by over-load of the proteosomal pathway for HIF1α degradation and the consequent accumulation of undegraded HIF1α. Accordingly, it is considered that modulation of SM20 and the related genes EGLN3 (BAB15101), c1orf12 (AAG34568), and CAB81622 may have applications in the treatment of diseases resulting from disturbances in proteosome function, such as prion diseases and other neuro-degenerative diseases.

These data provide the first connection between these related genes and the physiological response to hypoxia. Recently published research papers have identified that the protein products of these genes can

act as proline hydroxylases (see Bruick RK et al Science. 2001 294:1337-40 and Epstein AC et al Cell. 107:43-54). This is consistent with our observations that certain proline hydroxylases are induced in response to hypoxia and the genes EGLN1 and EGLN3 are part of the hypoxia response. For example, two genes encoding proline hydroxylases have been identified herein as being increased in expression in response to hypoxia (proline 4-hydroxylase, alpha polypeptide 1; SeqID: 231/232, proline 4-hydroxylase, alpha polypeptide II; SeqID: 349/ 350). This identified a functional significance of proline hydroxylation as a response to hypoxia.

Proline hydroxylase leads to degradation of HIF1α in normoxia (HIF regulates its own degradation – feedback). Hydroxylated HIF1α + VHL leads to ubquitination and consequent degradation of HIF1α by proteosome. The activity of the prolyl hydroxylase is 0_2-dependent, so under conditions of hypoxia, HIF1α is not hydroxylated efficiently and is stabilised. HIF1α protein thus accumulates to a high level. The hypoxia-induction of the prolyl hydroxylase ensures that when 0_2 concentration returns to normal, there is sufficient enzyme available to target this high level of HIF1α efficiently for rapid degradation.

Degradation of HIF1α is dependent on HIF1-induced transcription (i.e. is hypoxia inducible). Berra et al (FEBS Lett 2001 Feb 23;491(1-2):85-90) raises the specific hypothesis of an unknown hypoxia-inducible factor which targets HIF1a for proteosomal degradation. It appears reasonable to propose that this factor will clearly be hypoxia-inducible, to ensure that a rapid and effective constraint on the hypoxic response would operate on return to normoxia. It now appears as if the genes EGLN1 and EGLN3 form part of this mechanism.

It is also hypothesised that SM20 and the related genes EGLN3 (BAB15101), c1orf12 (AAG34568), and CAB81622 may act as tetramers. Known prolyl hydroxylases such as prolyl 4-hydroxylase (P4H) are known to act as tetramers of two alpha subunits and two beta subunits. SM20 and the related genes exhibits high similarity to the alpha subunit of P4H and it therefore seems likely that SM20 and the related genes are likely to have a binding partner that is equivalent to the beta subunit of P4H.

SM20 has been shown to bind to the transcription factor HIF1α, and shares a low level homology with a p53 binding protein. P53 is a transcription factor that is known to be involved in apoptosis. Accordingly, it is proposed that in addition to binding to HIF1A, SM20 and the related genes EGLN3 (BAB15101), c1orf12 (AAG34568), and CAB81622 may also bind and modify other transcription factors that are involved in the hypoxic response such as EPAS and HIF3A, or other transcription factors such as p53 and thereby influencing apoptosis. This aspect of the invention thus provides dimer and tetrameric forms of the EGLN3 (BAB15101), c1orf12 (AAG34568), and CAB81622 proteins, preferably complexed with a protein selected from the group consisting of HIF1α, p53 and a protein binding partner that is equivalent to the beta subunit of P4H. Preferably, such dimers and tetramers are heterodimers/heterotetramers.

To provide further evidence that these related genes are a significant part of the hypoxia response additional expression data is presented here.

Expression profiles for these two genes will be displayed with pre-chip normalisation to correct for differences in RNA labelling etc, but within each gene no further normalisation is done (per-gene normalisation), so the relative absolute expression levels of the two genes can be compared and Y-axis units between separate graphs from the same experiment are comparable. These graphs are presented as Figures 32b (c1orf12) and 32c (EGLN3).

It can be seen from these Figures that both genes (c1orf12 and EGLN3) are inducible in response to hypoxia in macrophages whether activated by gamma interferon and lipopolysaccharide or if de-activated by treatment with interleukin-10. In macrophages the absolute expression level of C1orf12 appears to be higher than EGLN3.

There is a prejudice in the art that the response to hypoxia is generic to all cell types. Contrary to this, we show herein that genes are regulated by hypoxia to a greater degree in certain cell types, substantiating their utility in designing specific therapeutic products for diseases involving those cell types.

From Figures 32a and 32d and the data presented below, differing expression profiles of the two related genes c1ORF12 and EGLN3 are apparent throughout the 11 tested cell types, though C1orf12 is generally expressed at higher levels than EGLN3.

Cell type	Oxygen	mRNA expression (c1ORF12 SeqID:89/90)	mRNA expression (EGLN3 SeqID:85/86)
adipocyte	normoxia	0.0075	0.0033
adipocyte	hypoxia 6hr	0.0091	0.0027
adipocyte	hypoxia 18hr	0.0182	0.0025
cardiomyocyte	normoxia	0.0067	0.0019
cardiomyocyte	hypoxia 6hr	0.0381	0.0023
cardiomyocyte	hypoxia 18hr	0.0201	0.0026
endothelial	normoxia	0.0198	0.0019
endothelial	hypoxia 6hr	0.0583	0.0033
endothelial	hypoxia 18hr	0.0397	0.0026
fibroblast	normoxia	0.0119	0.0032
fibroblast	hypoxia 6hr	0.0260	0.0046
fibroblast	hypoxia 18hr	0.0235	0.0040
hepatocyte	normoxia	0.0075	0.0080

hepatocyte	hypoxia 6hr	0.0074	0.0146
macrophage	normoxia	0.0033	0.0008
macrophage	hypoxia 6hr	0.0083	0.0018
macrophage	hypoxia 18hr	0.0058	0.0021
mammary epithelial	normoxia	0.0065	0.0014
mammary epithelial	hypoxia 6hr	0.0137	0.0055
mammary epithelial	hypoxia 18hr	0.0144	0.0065
monocyte	normoxia	0.0027	0.0006
monocyte	hypoxia 6hr	0.0084	0.0014
monocyte	hypoxia 18hr	0.0080	0.0016
neuroblastoma	normoxia	0.0344	0.0011
neuroblastoma	hypoxia 6hr	0.1085	0.0013
neuroblastoma	hypoxia 18hr	0.0551	0.0020
renal epithelial	normoxia	0.0275	0.0046
renal epithelial	hypoxia 6hr	0.0560	0.0046
renal epithelial	hypoxia 18hr	0.0395	0.0096
skeletal myocyte	normoxia	0.0088	0.0029
skeletal myocyte	hypoxia 6hr	0.0277	0.0035
skeletal myocyte	hypoxia 18hr	0.0245	0.0038

For instance, in the hypoxic hepatocyte (6hr) the normalised expression values of EGLN and c1orf12 are 0.015 and 0.0074 respectively, i.e. EGLN being the dominant gene. In contrast, in the neuroblastoma cell line SH-SY5Y, the normalised expression values of EGLN and c1orf12 after 6hr hypoxia are 0.0012 and 0.108 respectively, i.e. c1orf12 being the dominant gene by a large margin. This data demonstrates that c1ORF12 and EGLN3 are not constitutively expressed at an equal amount in different tissues indicating specificity of function. Therefore, it is considered that therapeutic products may be developed based on this data, with the goal of modulating proline hydroxylation of target proteins (such as HIF1alpha) in specific tissues, based on the differing expression profile of c1ORF12 and EGLN3 in those tissues.

In Example 1b herein, genes were identified from a custom array, which give a greater induction in macrophages (by a factor of at least 1.5) when hypoxia is augmented by over-expression of HIF1alpha or EPAS from an adenovirus. The data from the HIF/ EPAS over-expression work is presented herein in Example 1c, but specifically relating to c1ORF12 and EGLN3 is summarised in Figures 32e and 32f. From this data it is apparent that EGLN3/ FLJ21620 fis cl.COL07838 but not c1ORF12 is increased in expression by the transcription factor EPAS1 but not HIF1alpha. This is apparent by comparing

experimental condition 9 (hypoxia with EPAS overexpression; expression value=3.48) to that of 5 (hypoxia without EPAS overexpression; expression value= 1.65). This adds valuable information about the mechanism of regulation of the gene encoding EGLN3.

To confirm this data the RNA samples for experimental conditions 1,3,5,7,9 (corresponding to the high dose of adenovirus) were also measured using a different array-based methodology- the AffyMetrix GeneChip. The results of this experiment are presented in Figures 32g and 32h.

Functional Characterisation of EGL nine (C.elegans) homolog 3 role in the induction of Cardiomyocyte apoptotic cell death

EGLN3 has been cloned into pONY8.1 and Smart2.IRES.GFP equine infectious anaemia virus (EIAV) vectors, and AdCMV.TRACK.GFP (AdenoQuest) adenoviral genome vectors (see co-owned co-pending International patent application PCT/GB01/00758). These vectors have been used in "gain-of-function" studies in which EGLN3 has been overexpressed in order to elucidate corresponding protein function. Human embryo kidney (HEK 293T) and dog osteosarcoma (D17) cell lines have been used in transient plasmid transfection experiments to confirm EGLN3 expression from viral vector genomes. Rat cardiomyocyte cell line (H9C2) and primary human neonatal cardiomyocytes (PHNC) (BioWhittaker, CC2582) have been used in viral transduction experiments to determine the biological activity of EGLN3. In all cell types, expression of EGLN3 has been followed by combinations of immunofluorescence, Western blotting and TaqMan quantitative PCR. Immunofluorescence and Western blotting employ an antibody specific for the FLAG epitope engineered into the 3' terminus of EGL nine (C.elegans) homolog 3 (Sigma, F3165). TaqMan quantitative PCR utilises the SYBR Green method (Applied Biosystems).

Western blotting has confirmed the transient expression of EGLN3 from an EIAV genome construct in HEK 293T (expected size approx 717 bp, 26 Kda). Immunofluorescence has localised transient expression of EGL nine (C.elegans) homolog 3 from EIAV expression construct in HEK293T to the cytoplasm. Expression of EGL nine (C.elegans) homolog 3 is elevated after 4 hours exposure to hypoxic conditions (0.1% (v/v) oxygen), when compared to expression observed under normoxia (20% (v/v) oxygen) (see Figure 32i). TaqMan primers have been designed and optimised for the initial measurement of EGL nine (C.elegans) homolog 3 expression in EIAV or Adenovirus transduced H9C2 and PHNC (Forward: TCATCGACAGGCTGGTCCTC; Reverse: GTTCCATTTCCCGGATAGAA). All findings at the RNA level are corroborated by immunofluorescence and Western blotting analyses at the protein level.

EIAV transduction of H9C2 and PHNC has been optimised with constructs containing green fluorescence protein (GFP) and LacZ reporter genes, using the VSVg envelope and a range of MOI between 10 and 100. GFP results were scored by fluorescence microscopy, while LacZ transductants were identified

through the assay of β-galactosidase activity. An MOI of 50 transduced approximately 50% of the cell population.

EGLN3 is predicted to have pro-apoptotic activity in cardiomyocytes. Early, Mid and late phase apoptosis are characterised by translocation of membrane phospholipid phosphatidylserine (PS) from the inner face of the plasma membrane to the cell surface, activation of specific proteases (caspases) and fragmentation of DNA, respectively (Martin, S.J., et al., J. Exp. Med. 1995, 182, 1545-1556; Alnemri, E.S., et al., J. Cell. Biochem. 1997, 64, 33-42; Wylie, A.H., et al., Int. Rev. Cytol. 1980, 68, 251-306). Translocation of PS has been identified through use of ApoAlert kit (Clontech; K2025-1), which employs FITC-labelled antibodies to detect surface expression of the PS, Annexin V. Caspase activity has been followed using the homogeneous fluorimetric caspase assay (Roche; 3005372) which allows the quantification of caspase activity through the cleavage of a fluorescent substrate. DNA fragmentation has been estimated using the nuclear stain Hoescht 33345 (Sigma, B2261; and fluorescence microscopy to locate areas of chromatin condensation. Total viability of cell population has been quantified through measurement of the ability of mitochondrial reductase to metabolise the fluorescent substrate MTT (Sigma, M2128)(Levitz S.M & Diamond, R.D. J. Infect. Dis. 1985 Nov; 152(5):938-45).

Conditions for early, mid and late stage apoptosis in H9C2 and PHNC have been defined using hypoxia and nutrient-depleted growth medium to mimic those ischaemic conditions found *in vivo* (Brar, B.K., et al., J. Biol. Chem. 2000, 275, 8508-8514). Transduction of PHNC with EIAV vectors containing EGLN3 is sufficient to cause an increase in caspase activity in cells cultured under normoxic conditions, confirming the role of EGLN3 in the induction of cardiomyocyte apoptosis. Using an MOI of 50, a 2-fold increase in caspase activity was seen in EGLN3 transduced cells, when compared to controls 48 hours post transduction (see Figure 32j).

Increased expression of EGL nine (C.elegans) homolog 3 in transduced cells is confirmed by TaqMan, immunofluorescence and Western blotting. Similar experiments are performed to determine whether EGL nine (C.elegans) homolog 3 expression further sensitises H9C2 and PHNC to previously defined ischaemic insults. Staurosporine (Calbiochem; 569397) and Smart2.IRES.GFP EIAV vectors containing the Bax gene will be applied as chemical and viral pro-apoptotic controls, respectively (Yue, T-L., et al., J. Mol. Cell. Cardiol. 1998, 30, 495-507; Reed, J.C. J Cell Biol. 1994, 124(1-2):1-6).

Gene silencing approaches may be undertaken to down-regulate endogenous expression of EGLN3 in PHNC to determine the degree of protection against apoptotic cell death provided by a reduction in EGLN3 activity. RNA interference (RNAi) (Elbashir, SM et al., Nature 2001, 411, 494-498) is one method of sequence specific post-transcriptional gene silencing that may be employed. Short dsRNA oligonucleotides are synthesised *in vitro* and introduced into a cell. The sequence specific binding of

these dsRNA oligonucleotides triggers the degradation of target mRNA, reducing or ablating target protein expression. A Hammerhead ribozyme library, contained in EIAV expression vectors, may also be applied. Efficacy of both gene silencing approaches may be assessed initially through the measurement of EGLN3 expression, at the RNA level by TaqMan and at the protein level by Western blotting. Protection against previously described ischaemic insults provided by these methods of EGLN3 gene silencing may be assayed biologically as detailed above. Caspase inhibitors (caspase 3 inhibitor V, 2129002 and caspase inhibitor I, 627610, both Calbiochem) and Smart2.IRES.GFP EIAV vectors containing the Bcl-2 gene may be applied as chemical and viral anti-apoptotic controls, respectively (Kroemer, G. Nat Med. 1997, 3(6):614-20).

Similar "gain-of-function" and gene silencing approaches will be applied to the related gene, encoded by SEQ ID 90, named c1of12.

Genes with a greater response in renal epithelial cells

The dataset of Table 12 also contains genes which are induced preferentially in renal epithelial cells, in response to hypoxia. These genes are presented in Figures 39-44.

SeqID:117/118 EST
SeqID:129/130 Hypothetical protein FLJ22622
SeqID:31/32 TRIP-Br2
SeqID:301/302 Tumor protein D52
SeqID:91/92/92a Semaphorin 4b
SeqID:371/372 Dec-1

For Semaphorin 4b (SeqID:91/92/92a), the clone presented in Figure 43 is p1P14, corresponding to IMAGE clone acc BE910319, the sequence of which covers a large region of the gene including protein coding sequence, which may cross-hybridise to other members of the semaphorin family. A separate clone (p1D17) as found in the original filing, was derived from the subtracted library and corresponds to a more unique region of this gene in the untranslated region. From Table 12 it will be appreciated that a significant response is also found in the macrophage. This is validated by RNase protection assay data (see Figure 57). Further clarification of this gene using complementary experimentation methods will resolve the exact cell-type specific nature of the expression of this gene, though it is clear from this data that it is induced in renal epithelial cells and macrophages.

Genes with a greater response in mammary epithelial cells

The dataset of Table 12 also contains genes which are induced preferentially in mammary epithelial cells, in response to hypoxia. These genes are presented in Figures 45-52.

SeqID:447/448 Calgranulin A
SeqID:67/68 ERO1 (S. cerevisiae)-like
SeqID:25/26 Hypothetical protein FLJ20500
SeqID:229/230 N-myc downstream regulated
SeqID:387/388 Decidual protein induced by progesterone
SeqID:379/380 Integrin, alpha 5
SeqID:225/226 Tissue factor
SeqID:237/238 COX-2

In the case of Cox-2, which encodes a key drug target, it can be seen that in many cell types, especially the mammary epithelial cells, there is a clear induction in response to hypoxia. In contrast, for endothelial cells there is a very significant time-dependent *decrease* in Cox-2 gene expression in response to hypoxia. Similarly, for Calgranulin A, there is strong positive induction in hypoxic mammary epithelial cells, but in the macrophage, the response to hypoxia is negative. These clearly exemplify the unexpected finding that cell types respond to hypoxia differentially, both quantitatively but also qualitatively. This is not currently known.

Hypoxia regulation of Novel human genes

From Table 12, it will be appreciated that several genes with no prior annotation in public domain gene sequence databases are now identified as being regulated by hypoxia, in at least one cell type. To make this clear, these genes have been copied from Table 12 and presented in Tables 13 and 14), showing the hypoxia/ normoxia induction ratio of the cell type in which the response is most pronounced. These figures are derived by dividing the normalised expression value, as found in Table 5, in hypoxia by that in normoxia for the same cell type. In some cases, where hypoxia causes inhibition of gene expression, the fold change is prefixed by the term "DOWN". The cell type and time point of maximal response to hypoxia are also noted in Tables 13 and 14. The main purpose of Tables 13 and 14 is to demonstrate that these genes have significant responses to hypoxia *per se*.

In many cases, significant responses are seen in multiple cell types, though this data is not apparent here. In Table 13, the cDNA clones are currently un-annotated in public domain databases. In Table 14, the cDNA clones are currently annotated, but were not so as at the priority date.

Example 4: Additional disclosure of the effect of macrophage activation on hypoxia regulation of gene expression

In Example 2, it is shown that activated and resting macrophages respond to hypoxia in different ways, showing that the hypoxia response is not a generic phenomenon. To consolidate this data, experiments were performed with the custom array, using additional experimental conditions and with a more in-depth

analysis. Significantly, the expression values used are not simple hypoxia/ normoxia ratios, done separately for macrophages of differing activation status, but rather the values used allow comparison of the relative expression levels throughout the entire set of experimental conditions. Hence, for any gene, all values throughout the entire set of experimental conditions are calculated by comparison to the median level of that gene throughout the dataset. This allows a clearer appreciation of the effects of hypoxia in the context of cell activation status. The following data demonstrates that of the newly discovered genes responsive to hypoxia, expression changes are also seen in response to key cytokines of the immune system, implying functions outside of the generic response to hypoxia and metabolism. This especially applies to unannotated genes, including ESTs and hypothetical proteins, showing potential functions in inflammation and angiogenesis on the basis of cytokine-regulation.

Macrophages were derived and cultured as described elsewhere in the specification. A total of 6 experimental conditions were analysed, as shown below. Where cells were treated with cytokines or hypoxia (0.1% oxygen), this was for 6 hr. Lipopolysaccharide (LPS) (from *E.coli* 026:B6; Sigma), gamma Interferon (IFN) and Interleukin-10 (IL-10) were all used at a final concentration of 100ng/ml. The effect of gamma Interferon and Lipopolysaccharide is to activate macrophages, with a Th1 biased phenotype, as found in many inflammatory conditions. Interleukin-10 is a Th2 cytokine and de-activates macrophages, and suppresses their effector functions.

Experimental Condition		
1.	No cytokines	Normoxia
2.	No cytokines	Hypoxia
3.	IL-10	Normoxia
4.	IL-10	Hypoxia
5.	LPS+IFN	Normoxia
6.	LPS+IFN	Hypoxia

In Table 15, genes are shown which respond to LPS+IFN in normoxia by producing at least a 2-fold increase in expression, indicating probable pro-inflammatory functions. From this dataset various patterns of hypoxia regulation will be appreciated on top of the effect of LPS+IFN.

For instance, the gene SCYA8 (p1I21; SeqID: 479/480) is decreased in expression by hypoxia, changing from 0.54 to 0.18 between conditions #1 and #2. In condition #5 (LPS+IFN normoxia), expression is dramatically increased to a value of 19.6. When LPS+IFN is combined with hypoxia, this increase is dampened-down to a value of 12.2. So for this example, hypoxia and cell activation have opposing effects on gene expression. A similar expression profile is found for several other genes in Table 15.

In contrast, the gene P8 protein-candidate of metastasis 1 (p1F17; SeqID: 329/330) is increased in expression by hypoxia, changing from 0.26 to 1.78 between conditions #1 and #2. In condition #5 (LPS+IFN normoxia) expression is increased from condition #1 to a value of 1.16. In condition #6, (LPS+IFN normoxia) the expression is further increased to a value of 2.59. So for this example, hypoxia and cell activation have similar effects on expression (i.e. increases) and these are found to be synergistic. A similar expression profile is found for several other genes in Table 15, including for Semaphorin 4b (p1P14; SeqID:91/92/92a), which has been independently verified by RNase protection assay (see Figure 57).

A selection of novel genes taken from Table 15 is also presented as Figure 53. These novel genes are hence annotated here for the first time as being regulated not only by hypoxia, but also by Th1 inflammatory signals, as provided by LPS+IFN.

It will be appreciated that certain IMAGE clones were classed as novel and unannotated when the original patent filing was made (8 Dec 2000), but which can now be assigned to named genes. These are Uridine 5' monophosphate hydrolase 1 (clone p1I7; SeqID: 49/50) and Insulin induced protein 2 (clone p1D10; SeqID:75/76).

In Table 16, genes are shown which respond to LPS+IFN in normoxia by producing at least a 2-fold decrease in expression. From this dataset, various patterns of hypoxia regulation will be appreciated on top of the effect of LPS+IFN.

In Figure 54, novel genes from Table 16 which are down-regulated by LPS+IFN and up-regulated by hypoxia are presented. For most of these, the combined effect of LPS+IFN AND hypoxia produces only a minor induction above the level of expression for activated normoxic cells (for example p1F8/ SeqID:10/ Hypothetical Protein KIAA0914). In other cases, this is not the case, and hypoxia is able to over-ride the inhibitory effect of LPS+IFN on gene expression (for example p1D12/ SeqID:30/ Hypothetical Protein KIAA1376). This clearly demonstrates the finding that different cell types or physiological states of a cell type (as here), respond to hypoxia differently.

In Figure 55, novel genes from Table 16 which are down-regulated both by LPS+IFN and by hypoxia are presented. In many of the genes presented here, these stimuli are synergistic, with minimal expression obtained with a combination of LPS+IFN and hypoxia.

In Figure 56, a selection of named genes from Table 16 which are down-regulated by LPS+IFN, with various responses to hypoxia are presented. For the gene, Max-interacting Protein 1 two separate clones were available on the array corresponding to this gene (p1G5 from SeqID:280 and p1D22 from SeqID:120). In the original specification, the IMAGE clone corresponding to SeqID:120 (accession AA401496) was classified as an EST, and the IMAGE clone corresponding to SeqID:280 (accession

AA401496) was classified as "Max-interacting Protein 1", as determined by the UniGene database at that time. Now it is apparent that both of these clones correspond to Max-interacting Protein 1, explaining the similarity of their expression profiles in Figure 56. Clearly the response of this gene to hypoxia is inhibited by LPS+IFN.

The additional data showing effects of the Th1 activation stimulus LPS+IFN extends the finding of these genes as novel hypoxia regulated genes, and provides additional information about the relevance of these genes to disease mechanisms.

It will be appreciated that certain IMAGE clones were classed as novel and unannotated when the original patent filing was made (8 Dec 2000), but which can now be assigned to named genes. These are TRIP-Br2 (clone p1D15; SeqID:31/32), MAX-interacting protein 1 (clone p1D22; SeqID:119/120).

In Tables 15 and 16 and Figures 53-56, showing genes which respond to LPS+IFN, it will be noticed that some of these genes also response to the inhibitory cytokine IL-10 (e.g. Semaphorin 4b, Hypothetical protein CGI-117). Other genes respond only to IL-10, but not to LPS+IFN. Specific responses to IL-10 are significant because this cytokine has been shown to have utility in suppressing inflammatory reactions (Huizinga TW et al., *Rheumatology* 2000, 39: 1180-8).

Table 17 shows genes responsive to IL-10 (increased or decreased) but not affected significantly by LPS+IFN. Various patterns of hypoxia regulation will be appreciated.

Example 5: Gene expression in human tumors

One of the utilities of the genes identified herein relates to the diagnosis and treatment of human tumors, on the basis that hypoxia is frequently found in tumors.

A study has been performed to examine the expression of these genes in a selection of breast and ovary tumors, comparing expression with normal adjacent tissue from the same patient. There is expected to be a large degree of variation between different patients, and the study here contains only 5 patients with a range of diagnoses. Therefore although certain genes will be identified from this data, other genes in the current specification not flagged by this study are nevertheless likely to have utility in cancer.

Patients are designated as Letters:

E: 50 year old Caucasian female. Diagnosis: ovarian adenocarcinoma. Normal ovarian tissue derived from an age-matched separate individual.

F: 60 year old female. Diagnosis: poorly differentiated adenocarcinoma. Normal ovarian tissue derived from the same individual.

G: 41 year old female. Diagnosis: moderately-differentiated adenocarcinoma. Normal ovarian tissue derived from the same individual.

H: 40 year old female. Diagnosis: invasive ductal carcinoma. Normal breast tissue derived from the same individual.

K: 58 year old female. Diagnosis: invasive ductal carcinoma. Normal breast tissue derived from the same individual.

Data normalisation was done per-chip to correct for differences in labelling and hybridisation efficiency. Per-gene normalisation was done such that the expression values of each gene are relative to the median value of that gene throughout the series of samples. By comparing the expression values under normal (nor) and tumor (tum) for a single patient, differences in expression between the normal and malignant tissue of that patient can be inferred.

In Table 18 are genes which are up-regulated at least 3-fold in at least one patient, comparing the tumor tissue to the adjacent normal tissue.

In Table 19 are genes which are down-regulated at least 3-fold in at least one patient, comparing the tumor tissue to the adjacent normal tissue.

Example 6: Effects of inflammatory cytokines on hypoxia-regulated genes

Tumor necrosis factor alpha (TNFα) is a key pro-inflammatory cytokine both produced by and acting on the macrophage. The significance of TNFα to human disease is well established in the art. This is particularly the case in Rheumatoid arthritis and neutralising antibodies to TNFα have been reported to offer clinical utility. Because hypoxia is another pathological condition exerted on macrophages in the synovia of RA patients, synergistic effects of these two stimuli are highly relevant to the discovery of novel inflammatory targets expressed by the macrophage. To investigate this, primary human macrophages were exposed to either hypoxia (0.1% oxygen) or 100 ng/ml TNFα or to both for 6hr. The data shown below provides further credence to the utility of the encoded proteins as inflammatory targets in macrophages and applies to *any* disease where hypoxia and TNFα are co-incident.

Gene expression levels were measured and compared using the custom gene array. In data analysis per-gene normalisation was set up such that expression values represent the fold-change compared with the expression in untreated normoxic cells. Genes which are increased in expression in response to TNFα by at least 2-fold, in either normoxic or hypoxic cells, are shown in Table 20. Genes which are decreased in expression in response to TNFα by at least 2-fold, in either normoxic or hypoxic cells, are shown in Table 21.

Another inflammatory cytokine implicated in diseases where hypoxia is frequently found is Interleukin-17 (IL-17). For example, this cytokine has been shown to mediate inflammation and joint destruction in arthritis (Lubberts et al *J.Immunol* 2001 167:1004-1013). IL-17 has also been shown to stimulate macrophages to release other key pro-inflammatory cytokines (Jovanovic et al *J Immunol* 1998 160:3513-21). Therefore genes which respond to both hypoxia and IL-17 are especially likely to be relevant to disease processes and have utility in the design of therapeutic products. Genes which are increased in expression in response to IL-17 by at least 2-fold, in either normoxic or hypoxic cells, are shown in Table 22. Genes which are decreased in expression in response to IL-17 by at least 2-fold, in either normoxic or hypoxic cells, are shown in Table 23.

The cytokine IL-15 is implicated in several disease in which macrophages and hypoxia both feature as elements of the inflammatory state, such as in atherosclerosis (Wuttge DM et al *Am J Pathol.* 2001 159:417-23) and rheumatoid arthritis (McInnes IB et al *Immunol Today.* 1998 19:75-9). Although the main target of IL-15 is T-cells effects have also been shown on monocytes (Badolato R et al *Blood.* 1997 90:2804-9). Therefore genes which respond to both hypoxia and IL-15 are especially likely to be relevant to disease processes and have utility in the design of therapeutic products. Genes which are increased in expression in response to IL-15 by at least 2-fold, in either normoxic or hypoxic cells, are shown in Table 24. Genes which are decreased in expression in response to IL-15 by at least 2-fold, in either normoxic or hypoxic cells, are shown in Table 25.

Example 7: Rat foetal cardiomyocytes

Primary rat foetal cardiomyocytes provide an attractive experimental model for studying the responses of cardiac cells to ischaemia. Cells are obtained which are non-immortalised and which are seen to contract or beat in culture. It is of interest to examine how the responses of these cells to hypoxia (or related experimental conditions) compared and contrasts to other cell types. These other cell types might include those that are similarly sensitive to the effects of hypoxia (such as neurones) or might be cells that show a higher tolerance to hypoxia (such as macrophages). Experiments are performed in parallel for cardiomyocytes and other cell type(s). The responses of these specific cell types is then determined by hybridising labelled mRNA to microarrays. Alternative methods will include the construction of subtracted cDNA libraries for the individual treated cell types and assessing which genes are contained therein by sequencing.

Methods

Cardiomyocytes are harvested from heart ventricles of embryos aged E18 days, using a cell isolation kit (Neonatal cardiomyocyte isolation system; Worthington Biochemical Corporation, Lakewood, New

Jersey, 08701). They are seeded at $5x10^6$ cells/100cm diameter petri dish in DMEM/M199, 10% horse serum, 5% FCS, 1% penicillin, streptomycin, glutamine for 5 days at 37C. Media is changed during the 5 days.

Other cell types used for comparison with cardiomyocytes, are cultured according to their optimum conditions and/ or the standard routine. These cell types may include cardiomyocytes in a different physiological setting, such as in an intact beating heart, or a different developmental state of the cardiomyocyte, such as cardiomyoblast.

Identical seeded petri dishes are placed either in a standard tissue culture incubator (95% air/ 5% CO2) or in a hypoxia incubator (0.1% oxygen / 5% CO2 / 0.1% oxygen for 6 hours. This is done separately for both cardiomyocytes and the other cell type(s) to be compared. Other experimental conditions might more closely approximate ishemia, by incorporating components additional to hypoxia.

At the end of the exposure to hypoxia, cells are placed on a chilled platform, washed in cold PBS and total RNA is extracted using RNazol B (Tel-Test, Inc; distributed by Biogenesis Ltd) following the manufacturers instructions. Where appropriate, polyadenylated mRNA is extracted from the total RNA using a commercial kit following the manufacturers instructions (Promega; PolyATract mRNA isolation System IV).

Array hybridisations and construction/analysis of subtracted cDNA libraries are performed according to standard methods or as described elsewhere in this specification.

Example 8: Comparison of the hypoxic-responses between populations of rat primary cultured neurons by a subtraction cloning / array screening approach.

Different regions of the central nervous system display different sensitivities to hypoxia and to ischaemia. Susceptibility to tissue damage in this manner may occur as a result of intrinsic differences in gene expression between cells. To evaluate this hypothesis, primary cultures of rat neurons from different regions of the brain are established. Cultures are exposed to various experimental conditions which are pertinent to pathologies of the hypoxic/ischemic brain. These would include hypoxic insults as have been described, or to hypoxia/ischaemia where the conditions more closely approximate pathological ischemia. Either condition may be preceded by prior hypoxic-preconditioning, where transient exposure to hypoxia renders cells less sensitive to subsequent acute treatment. For all possible experimental treatments, a similar routine is performed for distinct neuron subtypes, in order to compare their responses. Such comparisons may be made by hybridizing labelled mRNA to microarrays or derivatives thereof. Alternatively subtracted libraries might be constructed individually for each treated neuron subtype, and clones which are confirmed to be changed in expression to be sequenced. The collection of genes arising from the different neuron subtypes will be compared.

Methods

Primary cultures are established according to standard procedures from embryonic rats aged from E14 to E18 (Dunnett SB, Bjorkland A (Eds.) 1992. Neural Transplantation, A Practical Approach. IRL Press). Isolated neurons include but are not limited to those from ventral mesencephalon, striatum, hippocampus, cerebellum, cerebral cortex, dorsal root ganglia and superior cervical ganglia.

Cells are maintained in culture for 3-14 days in humidified culture incubators at 37°C, 5% CO2, 95% air (Normoxia) in Neurobasal Medium (Brewer GJ, 1995, *Journal of Neuroscience Research* 42:674-83) supplemented with B27 (both Life Technologies). For the hypoxia-preconditioning, cells are transferred to a second incubator at 37°C, 5% CO2, 94.9% Nitrogen, 0.1% Oxygen (Hypoxia) for 30-180 minutes and returned to the normoxic incubator for 24 hours (Pringle *et al.*, 1997, *Neuropathology and Applied Neurobiology* 23:289-298). For the hypoxic stimulus, either independent from or subsequent to hypoxia-preconditioning, cells are transferred to the hypoxic incubator for 2-6 hours as determined in time course experiments. Additionally, as appropriate, the medium in which the cells are grown is replaced with glucose-free media for establishment of experimental ischaemia (Ray AM, Owen DE, Evans ML, Davis JB Benham, 2000. Caspase inhibitors are functionally neuroprotective against oxygen glucose deprivation induced CA1 death in rat organotypic hippocampal slices). At the end of the exposure to hypoxia (or hypoxia/ischaemia), cells are, placed on a chilled platform, washed in cold PBS and total RNA is extracted using RNazol B (Tel-Test, Inc; distributed by Biogenesis Ltd) following the manufacturers instructions. Where appropriate, polyadenylated mRNA is extracted from the total RNA using a commercial kit following the manufacturers instructions (Promega; PolyATract mRNA isolation System IV).

Array hybridisations and construction/analysis of subtracted cDNA libraries are performed according to standard methods or as described elsewhere in this specification.

Example 9: Semaphorin 4b

We have screened cDNA libraries derived from the human brain and leukocytes, to obtain an unequivocal and accurate full length cDNA sequence (SEQ ID No 92a) and the accurate presumptive amino acid sequence (SEQ ID No 91).

The amino acid sequence above was derived by taking the first ATG. We have various independent lines of evidence that this is the *bona fide* translation initiation codon.

Basic analysis of this sequence, reveals the following motifs:
signal peptide (pSORT) Start: 1 End: 37;
Transmembrane (pSORT) Start: 718 End: 734;
cleavage site (pSORT) Start: 38 End: 38;

Proline rich region Start: 758 End: 824;

Sema domain (pfam) Start: 70 End: 503;

Plexin repeat (pfam) Start: 525 End: 548;

integrin, beta domain (pfam) Start: 532 End: 546;

cytoplasmic tail Start: 735 End: 837.

To confirm the hypoxic regulation of Sema4b, we used RNase protection assay (see Figure 57). Hypoxia is a feature of several inflammatory conditions often accompanied by superoxide radicals and the immune regulator gamma interferon. In this experiment we have made the following findings:

- Expression is activated by hypoxia (3.3 fold)
- Expression is activated by gamma interferon and LPS (3.9 fold)
- Expression is activated synergistically by hypoxia plus gamma interferon/ LPS (7.3 fold)
- Expression is activated by superoxide radicals (5.0 fold)

To investigate the size of the mRNA and the tissue distribution, Northern blotting was done (see Figure 58). This shows that the gene is expressed as a single transcript at relatively low levels in unstimulated human tissues.

We have also found that a molecule that is probably associated with Semaphorin 4B, called psd-95 is another macrophage hypoxia-induced protein (see SEQ ID No 299). This is based on the fact that psd-95 binds the cytoplasmic tail of Sema4c (Inagaki et al., J Biol Chem. 2001; 276(12): 9174-81), which like Sema4b, contains proline rich sequence. Therefore, both Semaphorin 4B, and a probable partner are co-ordinately regulated by hypoxia.

Example 10: Discussion of relevance of individual clones

The Oxford BioMedica clone p1F12 represents Hypothetical protein FLJ13611. The protein sequence encoded by Hypothetical protein FLJ13611 is represented in the public databases by the accession NP_079217 and is described in this patent by Seq ID 1. The nucleotide sequence is represented in the public sequence databases by the accession NM_024941 and is described in this patent by Seq ID 2. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1F2 represents Hypothetical protein FLJ20037. The protein sequence encoded by Hypothetical protein FLJ20037 is represented in the public databases by the accession CAB65981 and is described in this patent by Seq ID 3. The nucleotide sequence is represented in the public sequence databases by the accession NM_017633 and is described in this patent by Seq ID 4. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore

implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Hypothetical protein FLJ20037 is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient. We expect increased activity of the gene product to have an anti-tumour effect.

The Oxford BioMedica clone p1F10 represents Hypothetical protein DKFZp434P0116. The protein sequence encoded by Hypothetical protein DKFZp434P0116 is represented in the public databases by the accession T46364 and is described in this patent by Seq ID 5. The nucleotide sequence is represented in the public sequence databases by the accession NM_017593 and is described in this patent by Seq ID 6. Hypothetical protein DKFZp434P0116 is predicted to be a kinase due to high structural similarity with other known kinases. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Hypothetical protein DKFZp434P0116 is repressed in macrophages activated by LPS and gamma interferon. We expect it to have an anti-inflammatory role.

The Oxford BioMedica clone p1F19 represents Hypothetical protein KIAA0212. The protein sequence encoded by Hypothetical protein KIAA0212 is represented in the public databases by the accession BAA13203 and is described in this patent by Seq ID 7. The nucleotide sequence is represented in the public sequence databases by the accession NM_014674 and is described in this patent by Seq ID 8. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1F8 represents Hypothetical protein KIAA0914. The protein sequence encoded by Hypothetical protein KIAA0914 is represented in the public databases by the accession NP_055698 and is described in this patent by Seq ID 9. The nucleotide sequence is represented in the public sequence databases by the accession NM_014883 and is described in this patent by Seq ID 10. Hypothetical protein KIAA0914 shows high structural similarity to Human Class I alpha 1,2-Mannosidase and conservation of active site and binding site residues, therefore we predict that Hypothetical protein KIAA0914 will act as a mannosidase. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Hypothetical protein

KIAA0914 is repressed in macrophages activated by LPS and gamma interferon. We expect the gene product to have an anti-inflammatory role.

The Oxford BioMedica clone p1F5 represents Hypothetical protein FLJ20281. The protein sequence encoded by Hypothetical protein FLJ20281 is represented in the public databases by the accession XP_008736 and is described in this patent by Seq ID 11. The nucleotide sequence is represented in the public sequence databases by the accession NM_017742 and is described in this patent by Seq ID 12. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. TNFalpha is an inflammatory cytokine, which acts on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. Hypothetical protein FLJ20281 is induced in macrophages activated by TNFalpha.

The Oxford BioMedica clone p1F18 represents Hypothetical protein KIAA0876. The protein sequence encoded by Hypothetical protein KIAA0876 is represented in the public databases by the accession BAA74899 and is described in this patent by Seq ID 13. The nucleotide sequence is represented in the public sequence databases by the accession XM_035625 and is described in this patent by Seq ID 14. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1F7 represents Spectrin, beta, non-erythrocytic 1. The protein sequence encoded by Spectrin, beta, non-erythrocytic 1 is represented in the public databases by the accession NP_003119 and is described in this patent by Seq ID 15. The nucleotide sequence is represented in the public sequence databases by the accession NM_003128 and is described in this patent by Seq ID 16. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Spectrin, beta, non-erythrocytic 1 is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient. We expect increased activity of the gene product to have an anti-tumour effect.

The Oxford BioMedica clone p1F21 represents Hematopoietic Zinc finger protein. The protein sequence encoded by Hematopoietic Zinc finger protein is represented in the public databases by the accession AAL08625 and is described in this patent by Seq ID 17. The nucleotide sequence is represented in the

public sequence databases by the accession AK024404 and is described in this patent by Seq ID 18. Hematopoietic Zinc finger protein is a transcriptional regulator that contains a Cys2-His2 zinc finger motif. It is predicted to bind to metal response elements (MRE) and therefore activate the transcription of genes that contain a MRE sequence within their promoter region such as metallothioneins. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. There is a prejudice in the art that the response to hypoxia is generic to all cell types. Contrary to this, we show that genes are regulated by hypoxia to a greater degree in certain cell types, substantiating their utility in designing specific therapeutic products for diseases involving those cell types. Monocytes and macrophages have been implicated in the following diseases involving hypoxia: rheumatoid arthritis, atherosclerosis, cancer, COPD. Hematopoietic Zinc finger protein is preferentially induced by hypoxia in monocytes or macrophages and a restricted number of other cell types. It is therefore a candidate for specific intervention for treatment or diagnosis of the above diseases.

The Oxford BioMedica clone p1F9 represents Hypothetical protein KIAA0742. The protein sequence encoded by Hypothetical protein KIAA0742 is represented in the public databases by the accession NP_060903 and is described in this patent by Seq ID 19. The nucleotide sequence is represented in the public sequence databases by the accession AB018285 and is described in this patent by Seq ID 20. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Hypothetical protein KIAA0742 is repressed in macrophages activated by LPS and gamma interferon. We expect it to have an anti-inflammatory role. Hypothetical protein KIAA0742 shows significant homolgy to the transcription factor hairless. We therefore propose that Hypothetical protein KIAA0742 may play a crucial role in the regulation of hair growth. Accordingly, this aspect of the invention includes the use of this protein, fragments and functional equivalents of this protein, encoding nucleic acid molecules, in addition to ligands that bind specifically to this protein, in the diagnosis and treatment of hair loss.

The Oxford BioMedica clone p1E13 represents Hypothetical protein PRO0823. The protein sequence encoded by Hypothetical protein PRO0823 is represented in the public databases by the accession AAF71073 and is described in this patent by Seq ID 21. The nucleotide sequence is represented in the public sequence databases by the accession AF116653 and is described in this patent by Seq ID 22. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore

implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Hypothetical protein PRO0823 is repressed in macrophages activated by LPS and gamma interferon. We expect it to have an anti-inflammatory role. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Hypothetical protein PRO0823 is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient. We expect increased activity of the gene product to have an anti-tumour effect.

The Oxford BioMedica clones p1D1 and p1D2 represent the Hypothetical protein FLJ10134. The protein sequence encoded by Hypothetical protein FLJ10134 is represented in the public databases by the accession NP_060474 and is described in this patent by Seq ID 23. The nucleotide sequence is represented in the public sequence databases by the accession NM_018004 and is described in this patent by Seq ID 24. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. HIF1alpha and EPAS1 are transcription factors that mediate the response to hypoxia of several genes, and have them selves been implicated in specific diseases. By adenoviral over-expression of EPAS1 we show augmentation of the hypoxic induction of certain genes, further confirming their status as responsive to hypoxia. Hypothetical protein FLJ10134 has been shown to be induced by hypoxia to a greater degree following adenoviral over-expression of EPAS1. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Hypothetical protein FLJ10134 is repressed in macrophages activated by LPS and gamma interferon. We expect it to have an anti-inflammatory role. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Hypothetical protein FLJ10134 is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient. We expect increased activity of the gene product to have an anti-tumour effect.

The Oxford BioMedica clone p1D4 represents Hypothetical protein FLJ20500. The protein sequence encoded by Hypothetical protein FLJ20500 is represented in the public databases by the accession NP_061931 and is described in this patent by Seq ID 25. The nucleotide sequence is represented in the public sequence databases by the accession NM_019058 and is described in this patent by Seq ID 26. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore

implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Hypothetical protein FLJ20500 is preferentially induced by hypoxia in mammary epithelial cells.

The Oxford BioMedica clone p1D9 represents Hypothetical protein DKFZP564D116. The protein sequence encoded by Hypothetical protein DKFZP564D116 is represented in the public databases by the accession T08708 and is described in this patent by Seq ID 27. The nucleotide sequence is represented in the public sequence databases by the accession AL050022 and is described in this patent by Seq ID 28. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Hypothetical protein DKFZP564D116 is repressed in macrophages activated by LPS and gamma interferon. TNFalpha is an inflammatory cytokine, which acts on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. Hypothetical protein DKFZP564D116 is induced in macrophages activated by TNFalpha.

The Oxford BioMedica clone p1D12 represents Hypothetical protein KIAA1376. The protein sequence encoded by Hypothetical protein KIAA1376 is represented in the public databases by the accession BAA92614 and is described in this patent by Seq ID 29. The nucleotide sequence is represented in the public sequence databases by the accession AB037797 and is described in this patent by Seq ID 30. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Hypothetical protein KIAA1376 is repressed in macrophages activated by LPS and gamma interferon. We expect it to have an anti-inflammatory role.

The Oxford BioMedica clone p1D15 represents TRIP-Br2. The protein sequence encoded by TRIP-Br2 is represented in the public databases by the accession NP_055570 and is described in this patent by Seq ID 31. The nucleotide sequence is represented in the public sequence databases by the accession NM_014755 and is described in this patent by Seq ID 32.. TRIP-BR2 is a PHD zinc finger and bromodomain interacting protein transcriptional regulator and is involved in the regulation of cell cycle

progression. Its hypoxia-regulation is likely to have important disease-relevant effects. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. The response of renal epithelial cells to hypoxia is pertinent to kidney failure, especially regarding the medullary tissue. TRIP-Br2 is preferentially induced by hypoxia in renal epithelial cells. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. TRIP-Br2 is repressed in macrophages activated by LPS and gamma interferon.

The Oxford BioMedica clone p1D16 represents Hypothetical protein FLJ20308. The protein sequence encoded by Hypothetical protein FLJ20308 is represented in the public databases by the accession XP_039852 and is described in this patent by Seq ID 33. The nucleotide sequence is represented in the public sequence databases by the accession AK000315 and is described in this patent by Seq ID 34. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Hypothetical protein FLJ20308 is repressed in macrophages activated by LPS and gamma interferon. We expect it to have an anti-inflammatory role.

The Oxford BioMedica clone p1J13 represents Hypothetical nuclear factor SBBI22. The protein sequence encoded by Hypothetical nuclear factor SBBI22 is represented in the public databases by the accession NP_065128 and is described in this patent by Seq ID 35. The nucleotide sequence is represented in the public sequence databases by the accession NM_020395 and is described in this patent by Seq ID 36. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1I22 represents Hypothetical protein KIAA1429. The protein sequence encoded by Hypothetical protein KIAA1429 is represented in the public databases by the accession BAA92667 and is described in this patent by Seq ID 37. The nucleotide sequence is represented in the public sequence databases by the accession AB037850 and is described in this patent by Seq ID 38. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1J6 represents Hypothetical protein FLJ10206. The protein sequence encoded by Hypothetical protein FLJ10206 is represented in the public databases by the accession AAH06108 and is described in this patent by Seq ID 39. The nucleotide sequence is represented in the public sequence databases by the accession NM_018025 and is described in this patent by Seq ID 40. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Hypothetical protein FLJ10206 is repressed in macrophages activated by IL-17 and is also repressed in macrophages activated by IL-15. These are pro-inflammatory cytokines, and we expect the hypothetical protein FLJ10206 to have an anti-inflammatory role.

The Oxford BioMedica clone p1I5 represents Hypothetical protein FLJ10815. The protein sequence encoded by Hypothetical protein FLJ10815 is represented in the public databases by the accession BAA91830 and is described in this patent by Seq ID 41. The nucleotide sequence is represented in the public sequence databases by the accession NM_018231 and is described in this patent by Seq ID 42. Hypothetical protein FLJ10815 is structurally similar to an alpha / beta barrel structure. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Hypothetical protein FLJ10815 is repressed in macrophages activated by LPS and gamma interferon. We expect it to have an anti-inflammatory role.

The Oxford BioMedica clone p1I13 represents Hypothetical protein FLJ11100. The protein sequence encoded by Hypothetical protein FLJ11100 is represented in the public databases by the accession NP_060701 and is described in this patent by Seq ID 43. The nucleotide sequence is represented in the public sequence databases by the accession NM_018321 and is described in this patent by Seq ID 44. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1I17 represents Hypothetical protein FLJ20644. The protein sequence encoded by Hypothetical protein FLJ20644 is represented in the public databases by the accession NP_060387 and is described in this patent by Seq ID 45. Hypothetical protein FLJ20644 is a putative

Serine/threonine phosphotase. Region 250 – 450 shows high structural similarity to other Serine/threonine phosphotases. The nucleotide sequence is represented in the public sequence databases by the accession NM_017917 and is described in this patent by Seq ID 46. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1I15 represents Hypothetical protein CGI-117. The protein sequence encoded by Hypothetical protein CGI-117 is represented in the public databases by the accession Q9Y3C1 and is described in this patent by Seq ID 47. The nucleotide sequence is represented in the public sequence databases by the accession NM_016391 and is described in this patent by Seq ID 48. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. HIF1alpha and EPAS1 are transcription factors that mediate the response to hypoxia of several genes, and have them selves been implicated in specific diseases. By adenoviral over-expression of HIF1alpha or EPAS1 we show augmentation of the hypoxic induction of certain genes, further confirming their status as responsive to hypoxia. Hypothetical protein CGI-117 has been shown to be induced by hypoxia to a greater degree following adenoviral over-expression of either HIF1alpha or EPAS1. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Hypothetical protein CGI-117 is repressed in macrophages activated by LPS and gamma interferon. We expect it to have an anti-inflammatory role.

The Oxford BioMedica clone p1I7 represents Uridine 5' monophosphate hydrolase 1. The protein sequence encoded by Uridine 5' monophosphate hydrolase 1 is represented in the public databases by the accession NP_057573 and is described in this patent by Seq ID 49. The nucleotide sequence is represented in the public sequence databases by the accession NM_016489 and is described in this patent by Seq ID 50. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Uridine 5' monophosphate hydrolase 1 is induced in macrophages activated by LPS and gamma interferon and is also is induced in macrophages activated by IL-15. We expect it to have a pro-inflammatory role, and its inhibition may have an anti-inflammatory effect.

The protein sequence encoded by Hypothetical protein KIAA0014 is represented in the public databases by the accession NP_055480 and is described in this patent by Seq ID 51. The nucleotide sequence is represented in the public sequence databases by the accession NM_014665 and is described in this patent by Seq ID 52. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1I4 represents Hypothetical protein HSPC196. The protein sequence encoded by Hypothetical protein HSPC196 is represented in the public databases by the accession NP_057548 and is described in this patent by Seq ID 53. The nucleotide sequence is represented in the public sequence databases by the accession NM_016464 and is described in this patent by Seq ID 54. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Hypothetical protein HSPC196 is repressed in macrophages activated by LPS and gamma interferon. We expect it to have an anti-inflammatory role.

The Oxford BioMedica clone p1I8 represents Hypothetical protein FLJ11296. The protein sequence encoded by Hypothetical protein FLJ11296 is represented in the public databases by the accession XP_004747 and is described in this patent by Seq ID 55. The nucleotide sequence is represented in the public sequence databases by the accession NM_018384 and is described in this patent by Seq ID 56. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1I16 represents Hypothetical protein KIAA1668. The protein sequence encoded by Hypothetical protein KIAA1668 is represented in the public databases by the accession BAB33338 and is described in this patent by Seq ID 57. The nucleotide sequence is represented in the public sequence databases by the accession AB051455 and is described in this patent by Seq ID 58. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1I11 represents SECIS binding protein 2. The protein sequence encoded by SECIS binding protein 2 is represented in the public databases by the accession AAK57518 and is

described in this patent by Seq ID 59. The nucleotide sequence is represented in the public sequence databases by the accession AF380995 and is described in this patent by Seq ID 60. SECIS binding protein 2 is a crucial component in the complex required for the translation of mammalian selenoprotein mRNAs. Selenoproteins are important responders to redox conditions and many selenoproteins are known to protect from cell death. Our demonstration of the hypoxia induction of SECIS binding protein 2 opens new avenues for diagnosis and therapeutic intervention. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, SECIS binding protein 2 is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient. We expect increased activity of the gene product to have an anti-tumour effect.

The Oxford BioMedica clone p1E8 represents cDNA: FLJ22249 fis, clone HRC02674. The sequence cDNA: FLJ22249 fis, clone HRC02674 is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession AK025902 and is described in this patent by Seq ID 62. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1E18 represents Plexin C1. The protein sequence encoded by Plexin C1 is represented in the public databases by the accession NP_005752 and is described in this patent by Seq ID 63. The nucleotide sequence is represented in the public sequence databases by the accession NM_005761 and is described in this patent by Seq ID 64. Plexins are a large family of receptors for transmembrane, secreted, and GPI-anchored semaphorins in vertebrates and play a significant role in signal transduction [Tamagnone et al 1999, Cell 99:71-80]. Elsewhere in this patent we disclose hypoxic regulation of a new semaphorin 4b, and we propose co-regulation of these molecules by hypoxia and their relevance to inflammatory disease, its diagnosis and therapy. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Plexin C1 is repressed in macrophages activated by LPS and gamma interferon.

The Oxford BioMedica clone p1E16 represents cDNA DKFZp586E1624. The sequence cDNA DKFZp586E1624 is not represented in the public databases by a protein accession. The nucleotide

sequence is represented in the public sequence databases by the accession AL110152 and is described in this patent by Seq ID 66. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. HIF1alpha and EPAS1 are transcription factors that mediate the response to hypoxia of several genes, and have them selves been implicated in specific diseases. By adenoviral over-expression of EPAS1 we show augmentation of the hypoxic induction of certain genes, further confirming their status as responsive to hypoxia. Its preferential regulation by EPAS1 provides a route to preferential intervention, to avoid toxicity to other tissues. The cDNA DKFZp586E1624 has been shown to be induced by hypoxia to a greater degree following adenoviral over-expression of EPAS1. Endothelial cells are key to angiogenesis, a process implicated in several diseases associated with hypoxia, including cancer and rheumatoid arthritis. The cDNA DKFZp586E1624 is preferentially induced by hypoxia in endothelial cells. We expect this gene product to have a pro-angiogenic effect, and its inhibition to have an anti-angiogenic effect.

The Oxford BioMedica clones p1D5 and p1D6 represent ERO1 (S. cerevisiae)-like. The protein sequence encoded by ERO1 (S. cerevisiae)-like is represented in the public databases by the accession NP_055399 and is described in this patent by Seq ID 67. The nucleotide sequence is represented in the public sequence databases by the accession NM_014584 and is described in this patent by Seq ID 68. ERO1 (S. cerevisiae)-like has been shown to be a flavin adenine dinucleotide (FAD) binding protein. Binding of FAD enables ERO1 (S. cerevisiae)-like to oxidise protein disulfide isomerase (PDI). We propose that the oxidisation of PDI by ERO1 (S. cerevisiae)-like stops PDI autodegradation, therefore increasing levels of the protein. Increased levels of PDI have been shown to be neuroprotective by inhibiting apoptotic cell death. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. HIF1alpha and EPAS1 are transcription factors that mediate the response to hypoxia of several genes, and have them selves been implicated in specific diseases. By adenoviral over-expression of EPAS1 we show augmentation of the hypoxic induction of certain genes, further confirming their status as responsive to hypoxia. ERO1 (S. cerevisiae)-like has been shown to be induced by hypoxia to a greater degree following adenoviral over-expression of EPAS1. Its preferential regulation by EPAS1 provides a route to preferential intervention, to avoid toxicity to other tissues. ERO1 (S. cerevisiae)-like is preferentially induced by hypoxia in mammary epithelial cells. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. ERO1 (S. cerevisiae)-like is repressed in macrophages activated by LPS and gamma interferon. Hypoxia is frequently found in human

tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, ERO1 (S. cerevisiae)-like is up-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1E12 represents Hypothetical protein DKFZP434E1723. The protein sequence encoded by Hypothetical protein DKFZP434E1723 is represented in the public databases by the accession XP_05338 and is described in this patent by Seq ID 69. The nucleotide sequence is represented in the public sequence databases by the accession BC010005 and is described in this patent by Seq ID 70. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1E10 represents cDNA FLJ11041 fis clone PLACE1004405. The sequence encoded by cDNA FLJ11041 fis, clone PLACE1004405 is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession AK001903 and is described in this patent by Seq ID 72. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. The cDNA FLJ11041 fis clone PLACE1004405 is induced in macrophages activated by LPS and gamma interferon. We expect it to have a pro-inflammatory role, and its inhibition may have an anti-inflammatory effect.

The Oxford BioMedica clone p1C21 represents Tubulin, beta, 4. The protein sequence encoded by Tubulin, beta, 4 is represented in the public databases by the accession NP_006077 and is described in this patent by Seq ID 73. The nucleotide sequence is represented in the public sequence databases by the accession NM_006086 and is described in this patent by Seq ID 74. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1D10 represents Insulin induced protein 2. The protein sequence encoded by Insulin induced protein 2 is represented in the public databases by the accession AAD43048 and is described in this patent by Seq ID 75. The nucleotide sequence is represented in the public sequence databases by the accession AF125392 and is described in this patent by Seq ID 76. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Insulin induced protein 2 is induced in macrophages activated by LPS and gamma interferon. We expect it to have a pro-inflammatory role,-and its inhibition may have an anti-inflammatory effect.

The Oxford BioMedica clones p1D13 and p1A22 represent Adenylate kinase 3. The protein sequence encoded by Adenylate kinase 3 is represented in the public databases by the accession NP_037542 and is described in this patent by Seq ID 77 and 263. The nucleotide sequence is represented in the public sequence databases by the accession NM_013410 and is described in this patent by Seq ID 78 and 264. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Adenylate kinase 3 is induced in macrophages activated by LPS and gamma interferon. TNFalpha is an inflammatory cytokine, which acts on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. Adenylate kinase 3 is induced in macrophages activated by TNFalpha.

The Oxford BioMedica clone p1E9 represents a novel PI-3-kinase adapter. The protein sequence encoded by the novel PI-3-kinase adapter is not represented in the public databases by a protein accession but is described in this patent by Seq ID 79. The nucleotide sequence of an unannotated EST corresponding to the novel PI-3-kinase adapter is represented in the public sequence databases by the accession R62339 and is described in this patent by Seq ID 80. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. There is a prejudice in the art that the response to hypoxia is generic to all cell types. Contrary to this, we show that genes are regulated by hypoxia to a greater degree in certain cell types, substantiating their utility in designing specific therapeutic products for diseases involving those cell types. Monocytes and macrophages have been implicated in the following diseases involving hypoxia: rheumatoid arthritis, atherosclerosis, cancer, COPD and peripheral arterial disease. The novel PI-3-kinase adapter is preferentially induced by hypoxia in monocytes or macrophages, indicating utility of the encoded protein in the design of therapeutic, prognostic and diagnostic products addressing diseases involving macrophages and hypoxia. In a gene array analysis it is

expressed in hypoxic monocytes and macrophages at levels 6-fold higher than the median expression level of this gene throughout 9 other cell types in either normoxia or hypoxia. In more sensitive TaqMan analysis the novel PI-3-kinase adapter it is found to be expressed at approximately 1000 times the levels of 9 other cell types, all exposed to hypoxia for 18hr. The relevance of the novel PI-3-kinase adapter to human disease is also appreciated from comparison with a related murine gene, BCAP. It is known that this gene is phosphorylated by the tyrosine kinase, Syk. We also show novel data regarding Syk, in that it is also induced in response to hypoxia in a tissue specific manner identical to that of the novel PI-3-kinase adapter. Therefore the biological relevance and utility of our discovery of hypoxic induction of the novel PI-3-kinase adapter gene is further highlighted.

The Oxford BioMedica clone p1F1 represents an unannotated EST. The protein sequence encoded by this EST is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession AA489477 and is described in this patent by Seq ID 82. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1E7 represents a novel Metallothionein. The protein sequence encoded by Novel Metallothionein is not represented in the public databases by a protein accession but is described in this patent by Seq ID 83. The nucleotide sequence is represented in the public sequence databases by the accession R06601 and is described in this patent by Seq ID 84. Metallothioneins can act as an antioxidant and free-radical scavenger and are therefore protective against cell death in hypoxia. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. HIF1alpha and EPAS1 are transcription factors that mediate the response to hypoxia of several genes, and have them selves been implicated in specific diseases. By adenoviral over-expression of HIF1alpha we show augmentation of the hypoxic induction of certain genes, further confirming their status as responsive to hypoxia. The novel Metallothionein represented by Seq ID 84 has been shown to be induced by hypoxia to a greater degree following adenoviral over-expression of HIF1alpha. Hepatocytes are the main cell type of the liver and genes that are induced in response to hypoxia in this cell type are relevant to development of diagnostics and therapeutics towards liver diseases involving hypoxia, including cirrhosis. The novel Metallothionein represented by Seq ID 84 is preferentially induced by hypoxia in hepatocytes. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been

shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. The novel Metallothionein represented by Seq ID 84 is induced in macrophages activated by LPS and gamma interferon.

The Oxford BioMedica clone p1E6 represents EGL nine (C.elegans) homolog 3. The protein sequence encoded by EGL nine (C.elegans) homolog 3 is represented in the public databases by the accession NP_071356 and is described in this patent by Seq ID 85. The nucleotide sequence is represented in the public sequence databases by the accession NM_022073 and is described in this patent by Seq ID 86. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. HIF1alpha and EPAS1 are transcription factors that mediate the response to hypoxia of several genes, and have them selves been implicated in specific diseases. By adenoviral over-expression of EPAS1 we show augmentation of the hypoxic induction of certain genes, further confirming their status as responsive to hypoxia. EGL nine (C.elegans) homolog 3 has been shown to be induced by hypoxia to a greater degree following adenoviral over-expression of EPAS1. Its preferential regulation by EPAS1 provides a route to preferential intervention, to avoid toxicity to other tissues. Hepatocytes are the main cell type of the liver and genes that are induced in response to hypoxia in this cell type are relevant to development of diagnostics and therapeutics towards liver diseases involving hypoxia, including cirrhosis. EGL nine (C.elegans) homolog 3 is preferentially induced by hypoxia in hepatocytes. We find that EGLN3 and a related human gene C1orf12 (seq ID 89/90) both of which are predicted to be proline hydroxylases, are expressed at differing absolute expression levels in different tissues. For instance, in the hypoxic hepatocyte (6hr) the normalised expression values of EGLN and c1orf12 are 0.015 and 0.0074 respectively, i.e. EGLN being the dominant gene. In contrast, in the neuroblastoma cell line SH-SY5Y, the normalised expression values of EGLN and c1orf12 after 6hr hypoxia are 0.0012 and 0.108 respectively, i.e. c1orf12 being the dominant gene by a large margin. This data demonstrates that c1ORF12 and EGLN3 are not constitutively expressed at an equal amount in different tissues indicating specificity of function. Therefore therapeutic products may be developed based on this data, with the goal of modulating proline hydroxylation of target proteins (such as HIF1alpha) in specific tissues, based on the differing expression profile of c1ORF12 and EGLN3 in those tissues.

The Oxford BioMedica clone p1D14 represents C1orf12. The protein sequence encoded by C1orf12 is represented in the public databases by the accession NP_071334 and is described in this patent by Seq ID 89. The nucleotide sequence is represented in the public sequence databases by the accession NM_022051 and is described in this patent by Seq ID 90. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. We find that C1orf12 and a related

human gene EGLN3 (seq ID 85/86) both of which are predicted to be proline hydroxylases, are expressed at differing absolute expression levels in different tissues. For instance, in the hypoxic hepatocyte (6hr) the normalised expression values of EGLN and c1orf12 are 0.015 and 0.0074 respectively, i.e. EGLN being the dominant gene. In contrast, in the neuroblastoma cell line SH-SY5Y, the normalised expression values of EGLN and c1orf12 after 6hr hypoxia are 0.0012 and 0.108 respectively, i.e. c1orf12 being the dominant gene by a large margin. This data demonstrates that c1ORF12 and EGLN3 are not constitutively expressed at an equal amount in different tissues indicating specificity of function. Therefore therapeutic products may be developed based on this data, with the goal of modulating proline hydroxylation of target proteins (such as HIF1alpha) in specific tissues, based on the differing expression profile of c1ORF12 and EGLN3 in those tissues.

The Oxford BioMedica clone p2B1 represents PRAME. The protein sequence encoded by PRAME is represented in the public databases by the accession NP_006106 and is described in this patent by Seq ID 87. The nucleotide sequence is represented in the public sequence databases by the accession NM_006115 and is described in this patent by Seq ID 88. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, PRAME is up-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient. PRAME is a well-known tumour-associated antigen. Our surprising demonstration of its hypoxia-regulation provides for an important diagnostic test to distinguish false-positive results. In addition, we show the relevance of PRAME to hypoxia-related functions of tumours such as angiogenesis.

The Oxford BioMedica clones p1D17 and p1P14 represent Semaphorin 4b. The protein sequence encoded by Semaphorin 4b is represented in the public databases by the accession BAB21836 and is described in this patent by Seq ID 91. The nucleotide sequence is represented in the public sequence databases by the accession AB051532 and is described in this patent by Seq ID 92. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. The response of renal epithelial cells to hypoxia is pertinent to kidney failure, especially regarding the medullary tissue. Semaphorin 4b is preferentially induced by hypoxia in renal epithelial cells. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Semaphorin 4b is induced in macrophages activated by LPS and gamma interferon.. Semaphorin 4b is also induced by

the the presence of reactive oxygen species. We expect it to have a pro-inflammatory role, and its inhibition may have an anti-inflammatory effect. We have cited elsewhere in this specification that a plexin is hypoxia-regulated, and we propose a functional relationship between these two molecules. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Semaphorin 4b is up-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient. Semaphorin 4b is also induced in response to superoxide radicals, as found in various disease states, implying utility. Semaphorin 4b is predicted to function in modulating several cellular processes key to human disease, including angiogenesis, inflammation, immune cell migration and tissue remodelling. Other Semaphorins including Semaphorin E, which are induced in response to hypoxia will also be implicated in these disease processes and have utility as described for Semaphorin 4b.

The Oxford BioMedica clone p1C24 represents SLC25A19. The protein sequence encoded by SLC25A19 is represented in the public databases by the accession NP_068380 and is described in this patent by Seq ID 93. The nucleotide sequence is represented in the public sequence databases by the accession NM_021734 and is described in this patent by Seq ID 94. SLC25A19 transports deoxynucleotides into mitochondria and is therefore essential for mtDNA synthesis. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1D3 represents Serine carboxypeptidase 1. The protein sequence encoded by Serine carboxypeptidase 1 is represented in the public databases by the accession NP_067639 and is described in this patent by Seq ID 95. The nucleotide sequence is represented in the public sequence databases by the accession NM_021626 and is described in this patent by Seq ID 96. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Serine carboxypeptidase 1 is repressed in macrophages activated by LPS and gamma interferon. TNFalpha is an inflammatory cytokine, which acts on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. Serine carboxypeptidase 1 is induced in macrophages activated by TNFalpha. Increased serine carboxypeptidase activity in glial cells has been shown to result in neurological abnormalities, due to the degradation of essential neuro-active factors.

Similarly, peripheral neurological disease could result from such activity in macrophages. Our demonstration of hypoxia regulation of serine carboxypeptidase activity opens a route for diagnosis and treatment of these diseases. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Serine carboxypeptidase 1 is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1E14 represents an unknown mRNA (schizophrenia-linked). The protein sequence encoded by the unknown mRNA (schizophrenia-linked) is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession AY010112 and is described in this patent by Seq ID 98. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. TNFalpha is an inflammatory cytokine, which acts on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. Unknown mRNA (schizophrenia-linked) is induced in macrophages activated by TNFalpha. There are many enzymic activities that can give rise to neurological abnormalities, and their hypoxia regulation is pertinent to the diagnosis and treatment of such diseases, including schizophrenia.

The Oxford BioMedica clone p1E20 represents Myo-inositol monophosphatase A3. The protein sequence encoded by Myo-inositol monophosphatase A3 is represented in the public databases by the accession AAK52336 and is described in this patent by Seq ID 99. The nucleotide sequence is represented in the public sequence databases by the accession NM_017813 and is described in this patent by Seq ID 100. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. As refered to elsewhere in this specification, we have found several components of the phosphatidylinisotol second messenger system to be hypoxia-regulated. This system has profound effects which are relevant to many diseases with known associations with hypoxia and ischaemia. Local and transient ischaemia is relevant to such diseases as rheumatoid arthritis and atherosclerosis, and also potentially to such diseases as schizophrenia and bi-polar disorder. It is instructive that lithium, which is a well-recognised treatment for affective disorders, appears to operate via the phosphatidylinisotol system [Pettegrew et al 2001, Bipolar Disord 3:189-201]. Macrophages are key to several diseases involving

hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Myo-inositol monophosphatase A3 is repressed in macrophages activated by LPS and gamma interferon.

The Oxford BioMedica clone p2A24 represents an unannotated EST. The protein sequence encoded by this EST is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession AA521314 and is described in this patent by Seq ID 102. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1E17 represents Hypothetical protein FLJ31668. The protein sequence encoded by Hypothetical protein FLJ31668 is represented in the public databases by the accession BAB71124 and is described in this patent by Seq ID 103. The nucleotide sequence is represented in the public sequence databases by the accession AK056230 and is described in this patent by Seq ID 104. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1E19 represents an unannotated EST. The protein sequence encoded by this EST is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession R51835 and is described in this patent by Seq ID 106. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, the EST represented by Seq ID 106 is up-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1E15 represents cDNA YI27F12. The protein sequence encoded by cDNA YI27F12 is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession AF075018 and is described in this patent by Seq ID 108. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. The cDNA YI27F12 is induced in macrophages treated with the inhibitory cytokine IL-10. The cDNA YI27F12 is repressed in macrophages activated by IL-17. We expect the product of cDNA YI27F12 to have an anti-inflammatory role.

The Oxford BioMedica clone p1E11 represents an unannotated EST. The protein sequence encoded by this EST is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession R69248 and is described in this patent by Seq ID 110. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1E23 represents cDNA FLJ14041 fis, clone HEMBA1005780. The protein sequence encoded by cDNA FLJ14041 fis, clone HEMBA1005780 is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession AK024103 and is described in this patent by Seq ID 112. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1E21 represents Glutamate-cysteine ligase, modifier subunit. The protein sequence encoded by Glutamate-cysteine ligase, modifier subunit is represented in the public databases by the accession NP_002052 and is described in this patent by Seq ID 113. The nucleotide sequence is represented in the public sequence databases by the accession NM_002061 and is described in this patent by Seq ID 114. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Glutamate-cysteine ligase is the rate-limiting enzyme of glutathione synthesis, and this enzyme is relevant to cell survival under stress. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Glutamate-cysteine ligase, modifier subunit is up-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1D23 represents PTEN. The protein sequence encoded by PTEN is represented in the public databases by the accession NP_000305 and is described in this patent by Seq ID 115. The nucleotide sequence is represented in the public sequence databases by the accession NM_000314 and is described in this patent by Seq ID 116. PTEN is a member of the mixed function, serine/threonine/tyrosine phosphatase subfamily of protein phosphatases. Its physiological substrates, however, are primarily 3-phosphorylated inositol phospholipids, which are products of phosphoinositide 3-kinases [Downes et al 2001, Biochem Soc Trans 29:846-51]. Hypoxia-regulation of this gene is a further element in the hypoxic regulation of this important second messenger system. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1D24 represents an unannotated EST. The protein sequence encoded by this EST is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession T73780 and is described in this patent by Seq ID 118. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. The response of renal epithelial cells to hypoxia is pertinent to kidney failure, especially regarding the medullary tissue. The EST represented by Seq ID 118 is preferentially induced by hypoxia in renal epithelial cells. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. The EST represented by Seq ID 118 is induced in macrophages activated by LPS and gamma interferon.

The Oxford BioMedica clones p1D22 and p1G5 represent MAX-interacting protein 1. The protein sequence encoded by MAX-interacting protein 1 is represented in the public databases by the accession NP_005953 and is described in this patent by Seq ID 119 and 279. The nucleotide sequence is represented in the public sequence databases by the accession NM_005962 and is described in this patent by Seq ID 120 and 280. MAX-interacting protein 1 is a negative regulator of myc oncoprotein with tumor suppressor properties. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. MAX-interacting protein 1 is repressed in macrophages activated by LPS and gamma interferon.

The Oxford BioMedica clone p1E2 represents Mannosidase, alpha, class 1A, member 1. The protein sequence encoded by Mannosidase, alpha, class 1A, member 1 is represented in the public databases by the accession NP_005898 and is described in this patent by Seq ID 121. The nucleotide sequence is represented in the public sequence databases by the accession NM_005907 and is described in this patent by Seq ID 122. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Mannosidase, alpha, class 1A, member 1 is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1E1 represents an unannotated EST. The protein sequence encoded by this EST is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession AA446361 and is described in this patent by Seq ID 124. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. The EST represented by Seq ID 124 is repressed in macrophages activated by LPS and gamma interferon.

The Oxford BioMedica clone p1E4 represents an unannotated EST. The protein sequence encoded by this EST is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession AA931411 and is described in this patent by Seq ID 126. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. The EST represented by Seq ID 126 is repressed in macrophages activated by LPS and gamma interferon. We expect this gene product to have an anti-inflammatory role. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, the EST represented by Seq ID 126 is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1D18 represents cDNA FLJ13443 fis, clone PLACE1002853. The protein sequence encoded by cDNA FLJ13443 fis, clone PLACE1002853 is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession AK023505 and is described in this patent by Seq ID 128. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. The cDNA FLJ13443 fis, clone PLACE1002853 is repressed in macrophages activated by LPS and gamma interferon. We expect it to have an anti-inflammatory role.

The Oxford BioMedica clone p1D21 represents Hypothetical protein FLJ22622. The protein sequence encoded by Hypothetical protein FLJ22622 is represented in the public databases by the accession BAB15424 and is described in this patent by Seq ID 129. The nucleotide sequence is represented in the public sequence databases by the accession NM_025151 and is described in this patent by Seq ID 130. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. The response of renal epithelial cells to hypoxia is pertinent to kidney failure, especially regarding the medullary tissue. Hypothetical protein FLJ22622 is preferentially induced by hypoxia in renal epithelial cells. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Hypothetical protein FLJ22622 is repressed in macrophages activated by LPS and gamma interferon. We expect it to have an anti-inflammatory role. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Hypothetical protein FLJ22622 is up-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1C22 represents CD84-H1. The protein sequence encoded by CD84-H1 is represented in the public databases by the accession AAK69052 and is described in this patent by Seq ID 131. The nucleotide sequence is represented in the public sequence databases by the accession AF275725 and is described in this patent by Seq ID 132. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1C23 represents Hypothetical protein FLJ12832. The protein sequence encoded by Hypothetical protein FLJ12832 is represented in the public databases by the accession XP_043394 and is described in this patent by Seq ID 133. The nucleotide sequence is represented in the public sequence databases by the accession AK022894 and is described in this patent by Seq ID 134. Hypothetical protien FLJ12832 is a putative ubiquitin as it shows high structural similarity to ubiquitin C and contains a ubiquitin domain. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1D11 represents an unannotated EST. The protein sequence encoded by this EST is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession AA251748 and is described in this patent by Seq ID 136. Hypoxia is an important feature of several diseases, and genes that respond to this

stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clones p1E3 and p1F16 represent CYP1B1. The protein sequence encoded by CYP1B1 is represented in the public databases by the accession NP_000095 and is described in this patent by Seq ID 137 and 325. The nucleotide sequence is represented in the public sequence databases by the accession NM_000104 and is described in this patent by Seq ID 138 and 326. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. There is a prejudice in the art that the response to hypoxia is generic to all cell types. Contrary to this, we show that genes are regulated by hypoxia to a greater degree in certain cell types, substantiating their utility in designing specific therapeutic products for diseases involving those cell types. Monocytes and macrophages have been implicated in the following diseases involving hypoxia: rheumatoid arthritis, atherosclerosis, cancer, COPD. CYP1B1 is preferentially induced by hypoxia in monocytes or macrophages and a restricted number of other cell types. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. CYP1B1 is repressed in macrophages activated by LPS and gamma interferon. TNFalpha is an inflammatory cytokine, which acts on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. CYP1B1 is induced in macrophages activated by TNFalpha. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, CYP1B1is up-regulated and also down regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1D20 represents Hypothetical protein KIAA1125. The protein sequence encoded by Hypothetical protein KIAA1125 is represented in the public databases by the accession XP_012932 and is described in this patent by Seq ID 139. The nucleotide sequence is represented in the public sequence databases by the accession AB032951 and is described in this patent by Seq ID 140. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1E5 represents Hepcidin antimicrobial peptide. The protein sequence encoded by Hepcidin antimicrobial peptide is represented in the public databases by the accession

NP_066998 and is described in this patent by Seq ID 141. The nucleotide sequence is represented in the public sequence databases by the accession NM_021175 and is described in this patent by Seq ID 142. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Hepatocytes are the main cell type of the liver and genes that are induced in response to hypoxia in this cell type are relevant to development of diagnostics and therapeutics towards liver diseases involving hypoxia, including cirrhosis. Hepcidin antimicrobial peptide is preferentially induced by hypoxia in hepatocytes. Hepcidin antimicrobial peptide is induced in macrophages treated with the inhibitory cytokine IL-10. TNFalpha is an inflammatory cytokine, which acts on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. Hepcidin antimicrobial peptide is repressed in macrophages activated by TNFalpha.

The Oxford BioMedica clone p1D19 represents an unannotated EST. The protein sequence encoded by this EST is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession R68736 and is described in this patent by Seq ID 144. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. The EST represented by Seq ID 144 is induced in macrophages activated by LPS and gamma interferon and is also induced in macrophages activated by IL-15. We expect the gene product relevant to the EST represented by Seq ID 144 to have a pro-inflammatory role, and its inhibition may have an anti-inflammatory effect.

The Oxford BioMedica clone p2A15 represents Sialyltransferase. The protein sequence encoded by Sialyltransferase is represented in the public databases by the accession NP_006447 and is described in this patent by Seq ID 145. The nucleotide sequence is represented in the public sequence databases by the accession NM_006456 and is described in this patent by Seq ID 146. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1I14 represents cDNA DKFZp564D016. The protein sequence encoded by cDNA DKFZp564D016 is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession AL050021 and is described in

this patent by Seq ID 148. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1I2 represents cDNA FLJ11302 fis, clone PLACE1009971. The protein sequence encoded by cDNA FLJ11302 fis, clone PLACE1009971 is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession AK002164 and is described in this patent by Seq ID 150. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. The cDNA FLJ11302 fis, clone PLACE1009971 is repressed in macrophages activated by LPS and gamma interferon. We expect it to have an anti-inflammatory role.

The Oxford BioMedica clone p1I12 represents Hypothetical protein MGC4549. The protein sequence encoded by Hypothetical protein MGC4549 is represented in the public databases by the accession XP_032794 and is described in this patent by Seq ID 151. The nucleotide sequence is represented in the public sequence databases by the accession NM_032377 and is described in this patent by Seq ID 152. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Hypothetical protein MGC4549 is induced in macrophages treated with the inhibitory cytokine IL-10. Hypothetical protein MGC4549 is repressed in macrophages activated by IL-17 and is also repressed in macrophages activated by IL-15. We expect it to have an anti-inflammatory role.

The Oxford BioMedica clone p1I3 represents ELMO2. The protein sequence encoded by ELMO2 is represented in the public databases by the accession AAL14467 and is described in this patent by Seq ID 153. The nucleotide sequence is represented in the public sequence databases by the accession XM_012933 and is described in this patent by Seq ID 154. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. This gene has been shown recently to promote phagocytosis and cell shape changes [Gumienny et al 2001, Cell 107:27-41]. These functions are typical of the macrophage, and are likely to play a role in macrophage-associated diseases.

The Oxford BioMedica clone p1I10 represents an unannotated EST. The protein sequence encoded by this EST is not represented in the public databases by a protein accession. The nucleotide sequence is

represented in the public sequence databases by the accession AA420992 and is described in this patent by Seq ID 156. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1H18 represents Ubiquitin specific protease 7. The protein sequence encoded by Ubiquitin specific protease 7 is represented in the public databases by the accession NP_003461 and is described in this patent by Seq ID 157. The nucleotide sequence is represented in the public sequence databases by the accession NM_003470 and is described in this patent by Seq ID 158. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Ubiquitin specific protease 7 is up-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient. We expect decreased activity of the gene product to have an anti-tumour effect.

The Oxford BioMedica clone p1H24 represents Nucleolar phosphoprotein Nopp34. The protein sequence encoded by Nucleolar phosphoprotein Nopp34 is represented in the public databases by the accession NP_115766 and is described in this patent by Seq ID 159. The nucleotide sequence is represented in the public sequence databases by the accession NM_032390 and is described in this patent by Seq ID 160. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1E22 represents cDNA FLJ13618 fis, clone PLACE1010925. The protein sequence encoded by cDNA FLJ13618 fis, clone PLACE1010925 is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession AK023680 and is described in this patent by Seq ID 162. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. The cDNA FLJ13618 fis, clone PLACE1010925 is induced in macrophages activated by LPS and gamma interferon.

The Oxford BioMedica clone p1H21 represents Hypothetical protein FLJ13511. The protein sequence encoded by Hypothetical protein FLJ13511 is represented in the public databases by the accession

NP_149014 and is described in this patent by Seq ID 163. The nucleotide sequence is represented in the public sequence databases by the accession NM_033025 and is described in this patent by Seq ID 164. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. There is a prejudice in the art that the response to hypoxia is generic to all cell types. Contrary to this, we show that genes are regulated by hypoxia to a greater degree in certain cell types, substantiating their utility in designing specific therapeutic products for diseases involving those cell types. Monocytes and macrophages have been implicated in the following diseases involving hypoxia: rheumatoid arthritis, atherosclerosis, cancer, COPD. Hypothetical protein FLJ13511 is preferentially induced by hypoxia in monocytes or macrophages and a restricted number of other cell types. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Hypothetical protein FLJ13511 is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1I1 represents Ribosomal RNA intergenic spacer. The protein sequence encoded by Ribosomal RNA intergenic spacer is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession AA664228 and is described in this patent by Seq ID 166. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1H14 represents an unannotated EST. The protein sequence encoded by this EST is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession R44397 and is described in this patent by Seq ID 168. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1H11 represents Carboxypeptidase M. The protein sequence encoded by Carboxypeptidase M is represented in the public databases by the accession NP_001865 and is described in this patent by Seq ID 169. The nucleotide sequence is represented in the public sequence databases by the accession NM_001874 and is described in this patent by Seq ID 170. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1H17 represents an unannotated EST. The protein sequence encoded by this EST is not represented in the public databases by a protein accession. The nucleotide sequence is

represented in the public sequence databases by the accession W87747 and is described in this patent by Seq ID 172. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, the EST represented by Seq ID 172 is up-regulated and also down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1H12 represents an unannotated EST. The protein sequence encoded by this EST is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession AA973568 and is described in this patent by Seq ID 174. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1H7 represents an unannotated EST. The protein sequence encoded by this EST is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession T98529 and is described in this patent by Seq ID 176. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1H15 represents an unannotated EST. The protein sequence encoded by this EST is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession AA022679 and is described in this patent by Seq ID 178. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, the EST represented by Seq ID 178 is up-regulated and also down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1H20 represents an unannotated EST. The protein sequence encoded by EST is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession H17921 and is described in this patent by Seq ID 180. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and

diagnostic products. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, the EST represented by Seq ID 180 is up-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient. We expect decreased activity of the gene product to have an anti-tumour effect.

The Oxford BioMedica clone p1H8 represents ABL. The protein sequence encoded by ABL is represented in the public databases by the accession NP_009297 and is described in this patent by Seq ID 181. The nucleotide sequence is represented in the public sequence databases by the accession NM_007313 and is described in this patent by Seq ID 182. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. ABL is induced in macrophages treated with the inhibitory cytokine IL-10. ABL is repressed in macrophages activated by IL-17 and is also repressed in macrophages activated by IL-15. We expect it to have an anti-inflammatory role Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, ABL is up-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1H16 represents an unannotated EST. The protein sequence encoded by this EST is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession W91958 and is described in this patent by Seq ID 184. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, the EST represented by Seq ID 184 is up-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1H9 represents an unannotated EST. The protein sequence encoded by this EST is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession R63694 and is described in this patent by Seq ID 186. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1H23 represents Hypothetical protein FLJ21094. The protein sequence encoded by Hypothetical protein FLJ21094 is represented in the public databases by the accession AAH14003 and is described in this patent by Seq ID 187. The nucleotide sequence is represented in the public sequence databases by the accession AK024747 and is described in this patent by Seq ID 188.

Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1H10 represents an unannotated EST. The protein sequence encoded by this EST is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession AA909912 and is described in this patent by Seq ID 190. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1H6 represents an unannotated EST. The protein sequence encoded by this EST is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession T99032 and is described in this patent by Seq ID 192. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. The EST represented by Seq ID 192 is induced in macrophages treated with the inhibitory cytokine IL-10. The EST represented by Seq ID 192 is repressed in macrophages activated by IL-15. We expect it to have an anti-inflammatory role.

The Oxford BioMedica clone p1H13 represents an unannotated EST. The protein sequence encoded by this EST is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession H52503 and is described in this patent by Seq ID 194. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. The EST represented by Seq ID 194 is repressed in macrophages activated by LPS and gamma interferon.

The Oxford BioMedica clone p1H19 represents an unannotated EST. The protein sequence encoded by this EST is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession AA127017 and is described in this patent by Seq ID 196. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Hypoxia is frequently found in human tumours where macrophage

infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, the EST represented by the Seq ID 196 is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1G22 represents an unannotated EST. The protein sequence encoded by this EST is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession R38647 and is described in this patent by Seq ID 198. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Endothelial cells are key to angiogenesis, a process implicated in several diseases associated with hypoxia, including cancer and rheumatoid arthritis. The EST represented by Seq ID 198 is preferentially induced by hypoxia in endothelial cells. We expect this gene product to have a pro-angiogenic effect, and its inhibition to have an anti-angiogenic effect.

The Oxford BioMedica clone p1G21 represents an unannotated EST. The protein sequence encoded by this EST is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession T87233 and is described in this patent by Seq ID 200. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1H1 represents Hypothetical protein FLJ10826. The protein sequence encoded by Hypothetical protein FLJ10826 is represented in the public databases by the accession BAB14226 and is described in this patent by Seq ID 201. The nucleotide sequence is represented in the public sequence databases by the accession NM_018233 and is described in this patent by Seq ID 202. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1G20 represents cDNA YO23H03. The protein sequence encoded by cDNA YO23H03 is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession AF075053 and is described in this patent by Seq ID 204. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and

cytokines are especially relevant. The cDNA YO23H03 is repressed in macrophages activated by LPS and gamma interferon.

The Oxford BioMedica clone p1H5 represents Hypothetical protein FLJ22690. The protein sequence encoded by Hypothetical protein FLJ22690 is represented in the public databases by the accession NP_078987 and is described in this patent by Seq ID 205. The nucleotide sequence is represented in the public sequence databases by the accession NM_024711 and is described in this patent by Seq ID 206. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Endothelial cells are key to angiogenesis, a process implicated in several diseases associated with hypoxia, including cancer and rheumatoid arthritis. Hypothetical protein FLJ22690 is preferentially induced by hypoxia in endothelial cells. We expect this gene product to have a pro-angiogenic effect, and its inhibition to have an anti-angiogenic effect. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Hypothetical protein FLJ22690 is induced in macrophages activated by IL-15.

The Oxford BioMedica clone p1G19 represents Mitochondrion sequence. The protein sequence encoded by Mitochondrion sequence is represented in the public databases by the accession AAH05845 and is described in this patent by Seq ID 207. The nucleotide sequence is represented in the public sequence databases by the accession BC005845 and is described in this patent by Seq ID 208. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, the Mitochondrion sequence represented by Seq ID 208 is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1H2 represents Fatty acid binding protein 5. The protein sequence encoded by Fatty acid binding protein 5 is represented in the public databases by the accession NP_001435 and is described in this patent by Seq ID 209. The nucleotide sequence is represented in the public sequence databases by the accession NM_001444 and is described in this patent by Seq ID 210. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. There is a prejudice in the art that the response to hypoxia is generic to all cell types. Contrary to this, we show that genes are regulated by hypoxia to a greater degree in certain cell types, substantiating their utility in designing specific therapeutic products for diseases involving those cell

types. Monocytes and macrophages have been implicated in the following diseases involving hypoxia: rheumatoid arthritis, atherosclerosis, cancer, COPD. Fatty acid binding protein 5 is preferentially induced by hypoxia in monocytes or macrophages. Crucially and very recently, Fatty acid binding protein 5 expressed in macrophages has been shown to play a very important role in the development of atherosclerotic plaques [Layne et al 2001, FASEB J 15:2733-5]. Our demonstration of hypoxic-regulation of this gene not only makes clear how this gene can participate in disease initiation and progression, but provides for a potential route to diagnosis and therapy of atherosclerosis. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. TNFalpha is an inflammatory cytokine, which acts on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. Fatty acid binding protein 5 is repressed in macrophages activated by TNFalpha.

The Oxford BioMedica clone p1G18 represents Mitochondrion sequence. The protein sequence encoded by Mitochondrion sequence is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession BC001612 and is described in this patent by Seq ID 212. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. The Mitochondrion sequence represented by Seq ID 212 is repressed in macrophages activated by LPS and gamma interferon.

The Oxford BioMedica clone p1H4 represents an unannotated EST. The protein sequence encoded by this EST is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession AA679939 and is described in this patent by Seq ID 214. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. The EST represented by Seq ID 214 is repressed in macrophages

activated by IL-17. We expect it to have an anti-inflammatory role. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, the EST represented by the Seq ID 214 is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1H3 represents an unannotated EST. The protein sequence encoded by this EST is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession AA630167 and is described in this patent by Seq ID 216. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, the EST represented by Seq ID 216 is up-regulated and also down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The protein sequence encoded by BCL2/adenovirus E1B 19kD-interacting protein 3-like is represented in the public databases by the accession NP_004322 and is described in this patent by Seq ID 217. The nucleotide sequence is represented in the public sequence databases by the accession NM_004331 and is described in this patent by Seq ID 218. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The protein sequence encoded by SLC2A1 is represented in the public databases by the accession NP_006507 and is described in this patent by Seq ID 219. The nucleotide sequence is represented in the public sequence databases by the accession NM_006516 and is described in this patent by Seq ID 220. SLC2A1 is a glucose transporter gene and is also known as GLUT1. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1P3 represents PDGFB. The protein sequence encoded by PDGFB is represented in the public databases by the accession NP_148937 and is described in this patent by Seq ID 221. The nucleotide sequence is represented in the public sequence databases by the accession NM_033016 and is described in this patent by Seq ID 222. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene

expression responses to both hypoxia and cytokines are especially relevant. PDGFB is induced in macrophages activated by LPS and gamma interferon.

The Oxford BioMedica clones p1A8 and p1A9 represent Lactate dehydrogenase A. The protein sequence encoded by Lactate dehydrogenase A is represented in the public databases by the accession NP_005557 and is described in this patent by Seq ID 223. The nucleotide sequence is represented in the public sequence databases by the accession NM_005566 and is described in this patent by Seq ID 224. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Lactate dehydrogenase A is repressed in macrophages activated by LPS and gamma interferon and is also repressed in macrophages activated by IL-15. TNFalpha is an inflammatory cytokine, which acts on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. Lactate dehydrogenase A is induced in macrophages activated by TNFalpha. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Lactate dehydrogenase A is up-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1B17 represents Tissue factor. The protein sequence encoded by Tissue factor is represented in the public databases by the accession NP_001984 and is described in this patent by Seq ID 225. The nucleotide sequence is represented in the public sequence databases by the accession NM_001993 and is described in this patent by Seq ID 226. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Tissue factor is preferentially induced by hypoxia in mammary epithelial cells. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. TNFalpha is an inflammatory cytokine, which acts on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. Tissue factor is induced in macrophages activated by TNFalpha. Tissue factor is the primary initiator of

blood coagulation with structural homology to the cytokine receptor family, and has been implicated in various vascular processes including metastasis, angiogenesis, and atherosclerosis. Our demonstration of hypoxic regulation leads to a clear undertanding of the possibility of intervention in disease by modulation of Tissue factor activity.

The Oxford BioMedica clone p1O20 represents VEGF. The protein sequence encoded by VEGF is represented in the public databases by the accession NP_003367 and is described in this patent by Seq ID 227. The nucleotide sequence is represented in the public sequence databases by the accession NM_003376 and is described in this patent by Seq ID 228. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, VEGF is up-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1B2 represents N-myc downstream regulated. The protein sequence encoded by N-myc downstream regulated is represented in the public databases by the accession NP_006087 and is described in this patent by Seq ID 229. The nucleotide sequence is represented in the public sequence databases by the accession NM_006096 and is described in this patent by Seq ID 230. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. N-myc downstream regulated is preferentially induced by hypoxia in mammary epithelial cells.

The Oxford BioMedica clone p1B3 represents Proline 4-hydroxylase, alpha polypeptide I. The protein sequence encoded by Proline 4-hydroxylase, alpha polypeptide I is represented in the public databases by the accession NP_000908 and is described in this patent by Seq ID 231. The nucleotide sequence is represented in the public sequence databases by the accession NM_000917 and is described in this patent by Seq ID 232. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Proline 4-hydroxylase, alpha polypeptide I is repressed in macrophages activated by LPS and gamma interferon. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Proline

4-hydroxylase, alpha polypeptide I is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The protein sequence encoded by BCL2/adenovirus E1B-interacting protein 3 is represented in the public databases by the accession NP_004043 and is described in this patent by Seq ID 233. The nucleotide sequence is represented in the public sequence databases by the accession NM_004052 and is described in this patent by Seq ID 234. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clones p1B18 and p1B19 represent Plasminogen activator inhibitor, type 1. The protein sequence encoded by Plasminogen activator inhibitor, type 1 is represented in the public databases by the accession NP_000593 and is described in this patent by Seq ID 235. The nucleotide sequence is represented in the public sequence databases by the accession NM_000602 and is described in this patent by Seq ID 236. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Plasminogen activator inhibitor, type 1 is induced in macrophages activated by LPS and gamma interferon. Plasminogen activator inhibitor, type 1 is repressed in macrophages activated by IL-17. TNFalpha is an inflammatory cytokine, which acts on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. Plasminogen activator inhibitor, type 1 is induced in macrophages activated by TNFalpha. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Plasminogen activator inhibitor, type 1 is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1N17 represents COX-2. The protein sequence encoded by COX-2 is represented in the public databases by the accession NP_000954 and is described in this patent by Seq ID 237. The nucleotide sequence is represented in the public sequence databases by the accession NM_000963 and is described in this patent by Seq ID 238. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. COX-2 is preferentially induced by hypoxia in mammary epithelial cells. Macrophages are key to several diseases involving hypoxia, and

contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. COX-2 is induced in macrophages activated by LPS and gamma interferon. TNFalpha is an inflammatory cytokine, which acts on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. COX-2 is induced in macrophages activated by TNFalpha. In view of the known role of COX-2 in prostaglandin synthesis and tumour progression, its induction by hypoxia has profound clinical implications, and clear utility in diagnosis and therapy. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found.

The Oxford BioMedica clone p1A24 represents Metallothionein 1H. The protein sequence encoded by Metallothionein 1H is represented in the public databases by the accession NP_005942 and is described in this patent by Seq ID 239. The nucleotide sequence is represented in the public sequence databases by the accession NM_005951 and is described in this patent by Seq ID 240. Metallothioneins can act as an antioxidant and free-radical scavenger and are therefore protective against cell death in hypoxia. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Hepatocytes are the main cell type of the liver and genes which are induced in response to hypoxia in this cell type are relevant to development of diagnostics and therapeutics towards liver diseases involving hypoxia, including cirrhosis. Metallothionein 1H is preferentially induced by hypoxia in hepatocytes. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Metallothionein 1H is induced in macrophages activated by LPS and gamma interferon.

The protein sequence encoded by Metallothionein 1L is represented in the public databases by the accession NP_002441 and is described in this patent by Seq ID 241. The nucleotide sequence is represented in the public sequence databases by the accession NM_002450 and is described in this patent by Seq ID 242. Metallothioneins can act as an antioxidant and free-radical scavenger and are therefore protective against cell death in hypoxia. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1B1 represents Metallothionein 1G. The protein sequence encoded by Metallothionein 1G is represented in the public databases by the accession NP_005941 and is described in this patent by Seq ID 243. The nucleotide sequence is represented in the public sequence databases by the

accession NM_005950 and is described in this patent by Seq ID 244. Metallothioneins can act as an antioxidant and free-radical scavenger and are therefore protective against cell death in hypoxia. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. HIF1alpha and EPAS1 are transcription factors which mediate the response to hypoxia of several genes, and have them selves been implicated in specific diseases. By adenoviral over-expression of HIF1alpha we show augmentation of the hypoxic induction of certain genes, further confirming their status as responsive to hypoxia. Metallothionein 1G has been shown to be induced by hypoxia to a greater degree following adenoviral over-expression of HIF1alpha. Hepatocytes are the main cell type of the liver and genes which are induced in response to hypoxia in this cell type are relevant to development of diagnostics and therapeutics towards liver diseases involving hypoxia, including cirrhosis. Metallothionein 1G is preferentially induced by hypoxia in hepatocytes. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Metallothionein 1G is induced in macrophages activated by LPS and gamma interferon. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Metallothionein 1G is up-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The protein sequence encoded by Metallothionein 1E (functional) is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession AA872383 and is described in this patent by Seq ID 246. Metallothioneins can act as an antioxidant and free-radical scavenger and are therefore protective against cell death in hypoxia. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clones p1A1, p1A2, p1A3 and p1A4 represent SLC2A3. The protein sequence encoded by SLC2A3 is represented in the public databases by the accession NP_008862 and is described in this patent by Seq ID 247. The nucleotide sequence is represented in the public sequence databases by the accession NM_006931 and is described in this patent by Seq ID 248. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. SLC2A3 is induced in macrophages treated with the inhibitory cytokine IL-10. TNFalpha is an inflammatory cytokine, which acts on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have

utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. SLC2A3 is induced in macrophages activated by TNFalpha.

The Oxford BioMedica clones p1A15, p1A16, p1A17 and p1A18 represent Hexokinase-2. The protein sequence encoded by Hexokinase-2 is represented in the public databases by the accession NP_000180 and is described in this patent by Seq ID 249. The nucleotide sequence is represented in the public sequence databases by the accession NM_000189 and is described in this patent by Seq ID 250. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Hexokinase-2 is repressed in macrophages activated by LPS and gamma interferon.

The Oxford BioMedica clones p1B14, p1B15 and p1B16 represent Interleukin 8. The protein sequence encoded by Interleukin 8 is represented in the public databases by the accession NP_000575 and is described in this patent by Seq ID 251. The nucleotide sequence is represented in the public sequence databases by the accession NM_000584 and is described in this patent by Seq ID 252. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Interleukin 8 is induced in macrophages activated by LPS and gamma interferon and is also induced in macrophages activated by IL-17. TNFalpha is an inflammatory cytokine, which acts on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. Interleukin 8 is induced in macrophages activated by TNFalpha. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Interleukin 8 is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clones p1A11 and p1A12 represent GAPDH. The protein sequence encoded by GAPDH is represented in the public databases by the accession NP_002037 and is described in this patent by Seq ID 253. The nucleotide sequence is represented in the public sequence databases by the accession NM_002046 and is described in this patent by Seq ID 254. Hypoxia is an important feature of several

diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. GAPDH is repressed in macrophages activated by LPS and gamma interferon and is also induced in macrophages activated by IL-17 or IL-15. TNFalpha is an inflammatory cytokine, which acts on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. GAPDH is induced in macrophages activated by TNFalpha. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, GAPDH is up-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1A13 represents Phosphoglycerate kinase 1. The protein sequence encoded by Phosphoglycerate kinase 1 is represented in the public databases by the accession NP_000282 and is described in this patent by Seq ID 255. The nucleotide sequence is represented in the public sequence databases by the accession NM_000291 and is described in this patent by Seq ID 256. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Phosphoglycerate kinase 1 is repressed in macrophages activated by LPS and gamma interferon. TNFalpha is an inflammatory cytokine, which acts on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. Phosphoglycerate kinase 1 is induced in macrophages activated by TNFalpha.

The Oxford BioMedica clone p1A14 represents Enolase 1. The protein sequence encoded by Enolase 1 is represented in the public databases by the accession NP_001419 and is described in this patent by Seq ID 257. The nucleotide sequence is represented in the public sequence databases by the accession NM_001428 and is described in this patent by Seq ID 258. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several

diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Enolase 1 is repressed in macrophages activated by LPS and gamma interferon. TNFalpha is an inflammatory cytokine, which acts on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. Enolase 1 is induced in macrophages activated by TNFalpha. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Enolase 1 is up-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1A19 represents Aldolase C. The protein sequence encoded by Aldolase C is represented in the public databases by the accession NP_005156 and is described in this patent by Seq ID 259. The nucleotide sequence is represented in the public sequence databases by the accession NM_005165 and is described in this patent by Seq ID 260. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Aldolase C is induced in macrophages activated by IL-15. Aldolase C is repressed in macrophages activated by IL-15. TNFalpha is an inflammatory cytokine, which acts on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. Aldolase C is induced in macrophages activated by TNFalpha. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Aldolase is up-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1A20 represents Triosephosphate isomerase 1. The protein sequence encoded by Triosephosphate isomerase 1 is represented in the public databases by the accession NP_000356 and is described in this patent by Seq ID 261. The nucleotide sequence is represented in the public sequence databases by the accession NM_000365 and is described in this patent by Seq ID 262. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic

products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Triosephosphate isomerase 1 is repressed in macrophages activated by LPS and gamma interferon and is also repressed in macrophages activated by IL-15. TNFalpha is an inflammatory cytokine, which acts on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. Triosephosphate isomerase 1 is induced in macrophages activated by TNFalpha.

The Oxford BioMedica clone p1A23 represents Metallothionein 2A. The protein sequence encoded by Metallothionein 2A is represented in the public databases by the accession NP_005944 and is described in this patent by Seq ID 265. The nucleotide sequence is represented in the public sequence databases by the accession NM_005953 and is described in this patent by Seq ID 266. Metallothioneins can act as an antioxidant and free-radical scavenger and are therefore protective against cell death in hypoxia. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. HIF1alpha and EPAS1 are transcription factors which mediate the response to hypoxia of several genes, and have them selves been implicated in specific diseases. By adenoviral over-expression of HIF1alpha we show augmentation of the hypoxic induction of certain genes, further confirming their status as responsive to hypoxia. Metallothionein 2A has been shown to be induced by hypoxia to a greater degree following adenoviral over-expression of HIF1alpha. Hepatocytes are the main cell type of the liver and genes which are induced in response to hypoxia in this cell type are relevant to development of diagnostics and therapeutics towards liver diseases involving hypoxia, including cirrhosis. Metallothionein 2A is preferentially induced by hypoxia in hepatocytes. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Metallothionein 2A is induced in macrophages activated by LPS and gamma interferon and also induced in macrophages activated by IL-15. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Metallothionein 2A is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clones p1B20 and p1B21 represent Osteopontin. The protein sequence encoded by Osteopontin is represented in the public databases by the accession NP_000573 and is described in this patent by Seq ID 267. The nucleotide sequence is represented in the public sequence databases by the

accession NM_000582 and is described in this patent by Seq ID 268. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. There is a prejudice in the art that the response to hypoxia is generic to all cell types. Contrary to this, we show that genes are regulated by hypoxia to a greater degree in certain cell types, substantiating their utility in designing specific therapeutic products for diseases involving those cell types. Monocytes and macrophages have been implicated in the following diseases involving hypoxia: rheumatoid arthritis, atherosclerosis, cancer, COPD. Osteopontin is preferentially induced by hypoxia in monocytes or macrophages and a restricted number of other cell types. Osteopontin has been shown recently to play a role in autoimmune disease [Chabas et al, 2001, Science 294: 1731-5]. We present a new association between the hypoxic response and autoimmune disease. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Osteopontin is repressed in macrophages activated by LPS and gamma interferon. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Osteopontin is up-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clones p1C17 and p1C18 represent Granulin. The protein sequence encoded by Granulin is represented in the public databases by the accession NP_002078 and is described in this patent by Seq ID 269. The nucleotide sequence is represented in the public sequence databases by the accession NM_002087 and is described in this patent by Seq ID 270. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Granulin is repressed in macrophages activated by LPS and gamma interferon. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Granulin is up-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient. The up-regulation of Granulin, which is a known growth factor, is a clinically significant feature of the hypoxic response with clear diagnostic and therapeutic utility.

The Oxford BioMedica clone p1D8 represents Hypoxia-inducible protein 2. The protein sequence encoded by Hypoxia-inducible protein 2 is represented in the public databases by the accession NP_037464 and is described in this patent by Seq ID 271. The nucleotide sequence is represented in the

public sequence databases by the accession NM_013332 and is described in this patent by Seq ID 272. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Hypoxia-inducible protein 2 is induced in macrophages treated with the inhibitory cytokine IL-10. Hypoxia-inducible protein 2 is repressed in macrophages activated by IL-17 and is also repressed in macrophages activated by IL-15.

The Oxford BioMedica clone p1A10 represents Enolase 2. The protein sequence encoded by Enolase 2 is represented in the public databases by the accession NP_001966 and is described in this patent by Seq ID 273. The nucleotide sequence is represented in the public sequence databases by the accession NM_001975 and is described in this patent by Seq ID 274. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Enolase 2 is repressed in macrophages activated by LPS and gamma interferon. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Enolase 2 is up-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient. We expect it to have an anti-inflammatory role.

The Oxford BioMedica clone p1G24 represents Glycogen synthase 1. The protein sequence encoded by Glycogen synthase 1 is represented in the public databases by the accession NP_002094 and is described in this patent by Seq ID 275. The nucleotide sequence is represented in the public sequence databases by the accession NM_002103 and is described in this patent by Seq ID 276. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Glycogen synthase 1 is repressed in macrophages activated by IL-17 and is also repressed in macrophages activated by IL-15.

The Oxford BioMedica clone p1G23 represents ALCAM. The protein sequence encoded by ALCAM is represented in the public databases by the accession NP_001618 and is described in this patent by Seq ID 277. The nucleotide sequence is represented in the public sequence databases by the accession NM_001627 and is described in this patent by Seq ID 278. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have

utility in the design of therapeutic, prognostic and diagnostic products. In view of the recently-discovered role of ALCAM in angiogenesis [Ohneda et al, 2001, Blood 2001 Oct 1;98(7):2134-42], our demonstration of hypoxic regulation of ALCAM has great clinical significance in the treatment and diagnosis of vascular disease and cancer.

The Oxford BioMedica clone p1G7 represents an unannotated EST. The protein sequence encoded by this EST is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession BC008022 and is described in this patent by Seq ID 282. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. The EST represented by Seq ID 282 is repressed in macrophages activated by LPS and gamma interferon. We expect the product of EST represented by Seq ID 282 to have an anti-inflammatory role.

The Oxford BioMedica clone p2A23 represents Chitinase 3-like 2. The protein sequence encoded by Chitinase 3-like 2 is represented in the public databases by the accession NP_003991 and is described in this patent by Seq ID 283. The nucleotide sequence is represented in the public sequence databases by the accession NM_004000 and is described in this patent by Seq ID 284. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Chitinase 3-like 2 is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1G1 represents BACH1. The protein sequence encoded by BACH1 is represented in the public databases by the accession NP_001177 and is described in this patent by Seq ID 285. The nucleotide sequence is represented in the public sequence databases by the accession NM_001186 and is described in this patent by Seq ID 286. BACH1, a novel helicase-like protein, interacts directly with BRCA1 and contributes to its DNA repair function. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. The induction by hypoxia of this known transcriptional repressor and potential oncogene [Cantor et al 2001, Cell 105:149-60] is a very significant finding with profound implications for the diagnosis and treatment of cancer.

The Oxford BioMedica clone p1G15 represents Phosphoglucomutase 1. The protein sequence encoded by Phosphoglucomutase 1 is represented in the public databases by the accession NP_002624 and is described in this patent by Seq ID 287. The nucleotide sequence is represented in the public sequence databases by the accession NM_002633 and is described in this patent by Seq ID 288. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Phosphoglucomutase 1 is induced in macrophages treated with the inhibitory cytokine IL-10.

The Oxford BioMedica clone p1F23 represents Hypothetical protein LOC51014. The protein sequence encoded by Hypothetical protein LOC51014 is represented in the public databases by the accession Q9Y3B3 and is described in this patent by Seq ID 289. The nucleotide sequence is represented in the public sequence databases by the accession AF151867 and is described in this patent by Seq ID 290. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Hypothetical protein LOC51014 is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1G8 represents Sin3-associated polypeptide. The protein sequence encoded by Sin3-associated polypeptide is represented in the public databases by the accession NP_003855 and is described in this patent by Seq ID 291. The nucleotide sequence is represented in the public sequence databases by the accession NM_003864 and is described in this patent by Seq ID 292. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1G13 represents ABCA1. The protein sequence encoded by ABCA1 is represented in the public databases by the accession NP_005493 and is described in this patent by Seq ID 293. The nucleotide sequence is represented in the public sequence databases by the accession NM_005502 and is described in this patent by Seq ID 294. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. ABCA1 is repressed in macrophages activated by LPS and gamma interferon. The hypoxia induction of ABCA1, which is known

to be relevant to atherosclerosis [Kielar et al 2001, Clin Chem 47:2089-97], has profound implications for the diagnosis and treatment of this disease.

The Oxford BioMedica clone p1G10 represents SEC24 member A. The protein sequence encoded by SEC24 member A is represented in the public databases by the accession CAA10334 and is described in this patent by Seq ID 295. The nucleotide sequence is represented in the public sequence databases by the accession AJ131244 and is described in this patent by Seq ID 296. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1F24 represents Glia-derived nexin. The protein sequence encoded by Glia-derived nexin is represented in the public databases by the accession AAA35883 and is described in this patent by Seq ID 297. The nucleotide sequence is represented in the public sequence databases by the accession M17783 and is described in this patent by Seq ID 298. Glia-derived nexin is a glycoprotein that functions as a serine protease inhibitor with activity towards thrombin, trypsin and urokinase. It is known to play a role in neuro-degeneration [Seidel et al 1998, Brain Res Mol Brain Res 60:296-300]. Thus the hypoxia induction of this gene is highly significant for the diagnosis and treatment of neuro-degenerative disease. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Glia-derived nexin is induced in macrophages activated by LPS and gamma interferon. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Glia-derived nexin is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1G2 represents Postsynaptic density-95. The protein sequence encoded by Postsynaptic density-95 is represented in the public databases by the accession NP_001356 and is described in this patent by Seq ID 299. The nucleotide sequence is represented in the public sequence databases by the accession NM_001365 and is described in this patent by Seq ID 300. Postsynaptic density-95 belongs to the MAGUK family of cell junction proteins. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. The recent demonstration for a possible role for Postsynaptic density-95 in ischaemic pre-conditioning [Tauskela et al 2001,

Neuroscience 107:571-584] underlines the medical significance of our determination of the hypoxic regulation of this gene, and its utility in the diagnosis and treatment of ischaemic disease.

The Oxford BioMedica clone p1G11 represents Tumour protein D52. The protein sequence encoded by Tumour protein D52 is represented in the public databases by the accession NP_005070 and is described in this patent by Seq ID 301. The nucleotide sequence is represented in the public sequence databases by the accession NM_005079 and is described in this patent by Seq ID 302. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. The response of renal epithelial cells to hypoxia is pertinent to kidney failure, especially regarding the medullary tissue. Tumour protein D52 is preferentially induced by hypoxia in renal epithelial cells. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Tumour protein D52 is up-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient. Our observation of hypoxia-regulation of this tumour-associated protein is highly significant for the diagnosis and treatment of cancer.

The Oxford BioMedica clone p1G16 represents p27, Kip1. The protein sequence encoded by p27, Kip1 is represented in the public databases by the accession NP_004055 and is described in this patent by Seq ID 303. The nucleotide sequence is represented in the public sequence databases by the accession NM_004064 and is described in this patent by Seq ID 304. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. The hypoxia regulation of this anti-mitogen has important utility in oncology and angiogenesis [Fouty et al 2001, Am J Respir Cell Mol Biol 25:652-658].

The Oxford BioMedica clone p1G9 represents PI-3-kinase, catalytic, beta polypeptide. The protein sequence encoded by PI-3-kinase, catalytic, beta polypeptide is represented in the public databases by the accession NP_006210 and is described in this patent by Seq ID 305. The nucleotide sequence is represented in the public sequence databases by the accession NM_006219 and is described in this patent by Seq ID 306. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. PI-3-kinase, catalytic, beta polypeptide is repressed in macrophages activated by LPS and gamma interferon. The very recent publication of a role for PI3 kinase in

angiogenesis induced by hypoxic pre-conditioning [Zhu et al 2001, FEBS Lett 508:369-74] re-enforces the clinical utility which we claim for this gene as a result of its hypoxia-induction.

The Oxford BioMedica clone p1G4 represents SLC5A3. The protein sequence encoded by SLC5A3 is represented in the public databases by the accession AAC39548 and is described in this patent by Seq ID 307. The nucleotide sequence is represented in the public sequence databases by the accession AF027153 and is described in this patent by Seq ID 308. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. SLC5A3 is over-expressed in the brains of children with Downs Syndrome, and may play a role in brain pathology [Berry et al 1999, J Pediatr 135:94-7]. Thus our claims of clinical utility following from hypoxia induction have great medical significance for the diagnosis and treatment of ischaemic and degenerative disease. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. SLC5A3 is repressed in macrophages activated by LPS and gamma interferon.

The Oxford BioMedica clone p1G14 represents Cytohesin binding protein. The protein sequence encoded by Cytohesin binding protein is represented in the public databases by the accession NP_004279 and is described in this patent by Seq ID 309. The nucleotide sequence is represented in the public sequence databases by the accession NM_004288 and is described in this patent by Seq ID 310. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Cytohesin has been shown to modulate PI3-kinase activity [Dierks et al 2001, J Biol Chem 276:37472-81], re-enforcing our claim here and elsewhere in this filing of the relevance to the hypoxic response of pathways controlled by the critical second-messenger PI3.

The Oxford BioMedica clones p1A5 and p1A6 represent SLC2A5. The protein sequence encoded by SLC2A5 is represented in the public databases by the accession NP_003030 and is described in this patent by Seq ID 311. The nucleotide sequence is represented in the public sequence databases by the accession NM_003039 and is described in this patent by Seq ID 312. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. SLC2A5 is repressed in macrophages activated by LPS and gamma interferon. Hypoxia is frequently found in human tumours

where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, SLC2A5 is up-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clones p1B6, p1B7, p1B8 and p1B9 represent Adipophilin. The protein sequence encoded by Adipophilin is represented in the public databases by the accession NP_001113 and is described in this patent by Seq ID 313. The nucleotide sequence is represented in the public sequence databases by the accession NM_001122 and is described in this patent by Seq ID 314. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. There is a prejudice in the art that the response to hypoxia is generic to all cell types. Contrary to this, we show that genes are regulated by hypoxia to a greater degree in certain cell types, substantiating their utility in designing specific therapeutic products for diseases involving those cell types. Monocytes and macrophages have been implicated in the following diseases involving hypoxia: rheumatoid arthritis, atherosclerosis, cancer, COPD. The hypoxia induction of adipophilin has profound implications for the causation, diagnosis and treatment of atherosclerosis, because this protein plays a key role in the uptake of lipid and foam cell formation [Buechler et al 2001, Biochim Biophys Acta 1532:97-104]. Adipophilin is preferentially induced by hypoxia in monocytes or macrophages and a restricted number of other cell types. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Adipophilin is repressed in macrophages activated by LPS and gamma interferon and is also repressed in macrophages activated by IL-15. TNFalpha is an inflammatory cytokine, which acts on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. Adipophilin is induced in macrophages activated by TNFalpha. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Adipophilin is up-regulated and also down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1G17 represents Early development regulator 2. The protein sequence encoded by Early development regulator 2 is represented in the public databases by the accession NP_004418 and is described in this patent by Seq ID 315. The nucleotide sequence is represented in the public sequence databases by the accession NM_004427 and is described in this patent by Seq ID 316. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore

implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Early development regulator 2 is repressed in macrophages activated by LPS and gamma interferon.

The Oxford BioMedica clone p1G3 represents B-cell translocation gene 1. The protein sequence encoded by B-cell translocation gene 1 is represented in the public databases by the accession NP_001722 and is described in this patent by Seq ID 317. The nucleotide sequence is represented in the public sequence databases by the accession NM_001731 and is described in this patent by Seq ID 318. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. B-cell translocation gene 1 is induced in macrophages activated by LPS and gamma interferon. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, B-cell translocation gene 1 is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1F22 represents Sorting nexin 9. The protein sequence encoded by Sorting nexin 9 is represented in the public databases by the accession NP_057308 and is described in this patent by Seq ID 319. The nucleotide sequence is represented in the public sequence databases by the accession NM_016224 and is described in this patent by Seq ID 320. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. TNFalpha is an inflammatory cytokine, which acts on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. Sorting nexin 9 is induced in macrophages activated by TNFalpha.

The Oxford BioMedica clone p1G12 represents Cyclin G2. The protein sequence encoded by Cyclin G2 is represented in the public databases by the accession NP_004345 and is described in this patent by Seq

ID 321. The nucleotide sequence is represented in the public sequence databases by the accession NM_004354 and is described in this patent by Seq ID 322. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Cyclin G2 is repressed in macrophages activated by LPS and gamma interferon.

The Oxford BioMedica clone p1F11 represents Hypothetical protein LOC51754. The protein sequence encoded by Hypothetical protein LOC51754 is represented in the public databases by the accession XP_049657 and is described in this patent by Seq ID 323. The nucleotide sequence is represented in the public sequence databases by the accession AL137430 and is described in this patent by Seq ID 324. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Hypothetical protein LOC51754 is repressed in macrophages activated by LPS and gamma interferon. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Hypothetical protein LOC51754 is up-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1F14 represents Butyrate response factor 1. The protein sequence encoded by Butyrate response factor 1 is represented in the public databases by the accession NP_004917 and is described in this patent by Seq ID 327. The nucleotide sequence is represented in the public sequence databases by the accession NM_004926 and is described in this patent by Seq ID 328. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. HIF1alpha and EPAS1 are transcription factors which mediate the response to hypoxia of several genes, and have them selves been implicated in specific diseases. By adenoviral over-expression of EPAS1 we show augmentation of the hypoxic induction of certain genes, further confirming their status as responsive to hypoxia. Butyrate response factor 1 has been shown to be induced by hypoxia to a greater degree following adenoviral over-expression of EPAS1. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer,

Butyrate response factor 1 is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1F17 represents P8 protein (candidate of metastasis 1). The protein sequence encoded by P8 protein (candidate of metastasis 1) is represented in the public databases by the accession NP_036517 and is described in this patent by Seq ID 329. The nucleotide sequence is represented in the public sequence databases by the accession NM_012385 and is described in this patent by Seq ID 330. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. P8 protein (candidate of metastasis 1) is induced in macrophages activated by LPS and gamma interferon. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, P8 protein (candidate of metastasis 1) is up-regulated and also down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clones p1C1 and p1C2 represent CXCR4. The protein sequence encoded by CXCR4 is represented in the public databases by the accession NP_003458 and is described in this patent by Seq ID 331. The nucleotide sequence is represented in the public sequence databases by the accession NM_003467 and is described in this patent by Seq ID 332. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. CXCR4 is repressed in macrophages activated by LPS and gamma interferon. TNFalpha is an inflammatory cytokine, which acts on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. CXCR4 is induced in macrophages activated by TNFalpha. CXCR4 may act through the PI3-K pathway. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, CXCR4 is up-regulated and also down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1F3 represents Hypothetical protein XP_017131. The protein sequence encoded by Hypothetical protein XP_017131 is represented in the public databases by the accession XP_017131 and is described in this patent by Seq ID 333. The nucleotide sequence is represented in the public sequence databases by the accession XM_017131 and is described in this patent by Seq ID 334. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Hypothetical protein XP_017131 is repressed in macrophages activated by LPS and gamma interferon. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Hypothetical protein XP_017131 is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1F20 represents Proline-rich protein with nuclear targeting signal. The protein sequence encoded by Proline-rich protein with nuclear targeting signal is represented in the public databases by the accession NP_006804 and is described in this patent by Seq ID 335. The nucleotide sequence is represented in the public sequence databases by the accession NM_006813 and is described in this patent by Seq ID 336. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. TNFalpha is an inflammatory cytokine, which acts on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. Proline-rich protein with nuclear targeting signal is induced in macrophages activated by TNFalpha. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Proline-rich protein with nuclear targeting signal is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1F6 represents Hypothetical protein hqp0376. The protein sequence encoded by Hypothetical protein hqp0376 is represented in the public databases by the accession T08745 and is described in this patent by Seq ID 337. The nucleotide sequence is represented in the public sequence databases by the accession AF078844 and is described in this patent by Seq ID 338.

Hypothetical protein hqp0376 is a putative dead box protein as it shows high structural similarity to dead box proteins and yeast initiation factor 4A. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. HIF1alpha and EPAS1 are transcription factors which mediate the response to hypoxia of several genes, and have them selves been implicated in specific diseases. By adenoviral over-expression of HIF1alpha we show augmentation of the hypoxic induction of certain genes, further confirming their status as responsive to hypoxia. Hypothetical protein hqp0376 has been shown to be induced by hypoxia to a greater degree following adenoviral over-expression of HIF1alpha. Hepatocytes are the main cell type of the liver and genes which are induced in response to hypoxia in this cell type are relevant to development of diagnostics and therapeutics towards liver diseases involving hypoxia, including cirrhosis. Hypothetical protein hqp0376 is preferentially induced by hypoxia in hepatocytes. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Hypothetical protein hqp0376 is induced in macrophages activated by LPS and gamma interferon.

The Oxford BioMedica clone p1F4 represents CYP1. The protein sequence encoded by CYP1 is represented in the public databases by the accession NP_000776 and is described in this patent by Seq ID 339. The nucleotide sequence is represented in the public sequence databases by the accession NM_000785 and is described in this patent by Seq ID 340. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. There is a prejudice in the art that the response to hypoxia is generic to all cell types. Contrary to this, we show that genes are regulated by hypoxia to a greater degree in certain cell types, substantiating their utility in designing specific therapeutic products for diseases involving those cell types. Monocytes and macrophages have been implicated in the following diseases involving hypoxia: rheumatoid arthritis, atherosclerosis, cancer, COPD. CYP1 is preferentially induced by hypoxia in monocytes or macrophages. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. CYP1 is induced in macrophages activated by LPS and gamma interferon. TNFalpha is an inflammatory cytokine, which acts on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have

utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. CYP1 is induced in macrophages activated by TNFalpha.

The Oxford BioMedica clone p1F15 represents SHB adaptor protein. The protein sequence encoded by SHB adaptor protein is represented in the public databases by the accession NP_003019 and is described in this patent by Seq ID 341. The nucleotide sequence is represented in the public sequence databases by the accession NM_003028 and is described in this patent by Seq ID 342. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. SHB adaptor protein participates in tyrosine kinase-mediated signalling and the regulation of angiogenesis and apotosis [Dixelius J. 2000, Blood 95:3403-11]. Our surprising observation of the hypoxia regulation of this protein has clear medical utility in the diagnosis and treatment of vascular disease and cancer. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. SHB adaptor protein is repressed in macrophages activated by LPS and gamma interferon.

The Oxford BioMedica clone p1F13 represents Papillomavirus regulatory factor PRF-1. The protein sequence encoded by Papillomavirus regulatory factor PRF-1 is represented in the public databases by the accession NP_061130 and is described in this patent by Seq ID 343. The nucleotide sequence is represented in the public sequence databases by the accession AK023418 and is described in this patent by Seq ID 344. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Papillomavirus regulatory factor PRF-1 is repressed in macrophages activated by LPS and gamma interferon.

The Oxford BioMedica clone p1A7 represents SLC31A2. The protein sequence encoded by SLC31A2 is represented in the public databases by the accession NP_001851 and is described in this patent by Seq ID 345. The nucleotide sequence is represented in the public sequence databases by the accession NM_001860 and is described in this patent by Seq ID 346. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene

expression responses to both hypoxia and cytokines are especially relevant. SLC31A2 is induced in macrophages activated by LPS and gamma interferon.

The Oxford BioMedica clone p1A21 represents UDP-glucose pyrophosphorylase 2. The protein sequence encoded by UDP-glucose pyrophosphorylase 2 is represented in the public databases by the accession NP_006750 and is described in this patent by Seq ID 347. The nucleotide sequence is represented in the public sequence databases by the accession NM_006759 and is described in this patent by Seq ID 348. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clones p1B4 and p1B5 represent Proline 4-hydroxylase, alpha polypeptide II. The protein sequence encoded by Proline 4-hydroxylase, alpha polypeptide II is represented in the public databases by the accession NP_004190 and is described in this patent by Seq ID 349. The nucleotide sequence is represented in the public sequence databases by the accession NM_004199 and is described in this patent by Seq ID 350. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Proline 4-hydroxylase, alpha polypeptide II is repressed in macrophages activated by LPS and gamma interferon and is also repressed in macrophages activated by IL-15. TNFalpha is an inflammatory cytokine, which acts on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. Proline 4-hydroxylase, alpha polypeptide II is induced in macrophages activated by TNFalpha. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Proline 4-hydroxylase, alpha polypeptide II is up-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clones p1B10, p1B11 and p1B12 represent Stearoyl-CoA desaturase. The protein sequence encoded by Stearoyl-CoA desaturase is represented in the public databases by the accession NP_005054 and is described in this patent by Seq ID 351. The nucleotide sequence is represented in the public sequence databases by the accession NM_005063 and is described in this patent by Seq ID 352. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic

products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. TNFalpha is an inflammatory cytokine, which acts on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. Stearoyl-CoA desaturase is induced in macrophages activated by TNFalpha.

The Oxford BioMedica clone p1B13 represents Diacylglycerol kinase, zeta. The protein sequence encoded by Diacylglycerol kinase, zeta is represented in the public databases by the accession NP_003637 and is described in this patent by Seq ID 353. The nucleotide sequence is represented in the public sequence databases by the accession NM_003646 and is described in this patent by Seq ID 354. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1B22 represents Protease, serine, 11. The protein sequence encoded by Protease, serine, 11 is represented in the public databases by the accession NP_002766 and is described in this patent by Seq ID 355. The nucleotide sequence is represented in the public sequence databases by the accession NM_002775 and is described in this patent by Seq ID 356. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Protease, serine, 11 is repressed in macrophages activated by LPS and gamma interferon. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Protease, serine, 11 is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1B23 represents Interleukin 1 receptor antagonist. The protein sequence encoded by Interleukin 1 receptor antagonist is represented in the public databases by the accession NP_000568 and is described in this patent by Seq ID 357. The nucleotide sequence is represented in the public sequence databases by the accession NM_000577 and is described in this patent by Seq ID 358. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic

products. There is a prejudice in the art that the response to hypoxia is generic to all cell types. Contrary to this, we show that genes are regulated by hypoxia to a greater degree in certain cell types, substantiating their utility in designing specific therapeutic products for diseases involving those cell types. Monocytes and macrophages have been implicated in the following diseases involving hypoxia: rheumatoid arthritis, atherosclerosis, cancer, COPD. Interleukin 1 receptor antagonist is preferentially induced by hypoxia in monocytes or macrophages. TNFalpha is an inflammatory cytokine, which acts on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. Interleukin 1 receptor antagonist is induced in macrophages activated by TNFalpha.

The Oxford BioMedica clone p1B24 represents NS1-binding protein. The protein sequence encoded by NS1-binding protein is represented in the public databases by the accession NP_006460 and is described in this patent by Seq ID 359. The nucleotide sequence is represented in the public sequence databases by the accession NM_006469 and is described in this patent by Seq ID 360. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1C3 represents Activin A receptor, type I. The protein sequence encoded by Activin A receptor, type I is represented in the public databases by the accession NP_001096 and is described in this patent by Seq ID 361. The nucleotide sequence is represented in the public sequence databases by the accession NM_001105 and is described in this patent by Seq ID 362. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Activin A is known to induce apoptosis [Hughes et al 1999, Prog Neurobiol 57:421-50], and so the regulation of its receptor by hypoxia has clear clinical significance. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Activin A receptor, type I is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1C4 represents FGF receptor activating protein 1. The protein sequence encoded by FGF receptor activating protein 1 is represented in the public databases by the accession NP_055304 and is described in this patent by Seq ID 363. The nucleotide sequence is represented in the public sequence databases by the accession NM_014489 and is described in this patent by Seq ID 364. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. FGF has been shown to enhance survival of cardiac cells after ischaemic insult [Sheikh et al

2001, Am J Physiol Heart Circ Physiol 280:H1039-50], and so our observation of the hypoxia-regulation of the FGF receptor activating protein 1 is highly significant for the diagnosis and treatment of ischaemic disease. FGF receptor activating protein 1 is induced in macrophages treated with the inhibitory cytokine IL-10. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, FGF receptor activating protein 1 is up-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1C5 represents Galectin 8. The protein sequence encoded by Galectin 8 is represented in the public databases by the accession NP_006490 and is described in this patent by Seq ID 365. The nucleotide sequence is represented in the public sequence databases by the accession NM_006499 and is described in this patent by Seq ID 366. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Galectin 8 is an important tumour marker [for review see Bidon et al 2001, Int J Mol Med 8:245-50], and so its hypoxia-regulation is highly significant clinically.

The Oxford BioMedica clone p1C6 represents Glucose phosphate isomerase. The protein sequence encoded by Glucose phosphate isomerase is represented in the public databases by the accession NP_000166 and is described in this patent by Seq ID 367. The nucleotide sequence is represented in the public sequence databases by the accession NM_000175 and is described in this patent by Seq ID 368. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Glucose phosphate isomerase is induced in macrophages activated by IL-17 and also induced in macrophages activated by IL-15. TNFalpha is an inflammatory cytokine, which acts on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. Glucose phosphate isomerase is induced in macrophages activated by TNFalpha. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Glucose phosphate isomerase is up-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1C7 represents D123. The protein sequence encoded by D123 is represented in the public databases by the accession NP_006014 and is described in this patent by Seq ID 369. The nucleotide sequence is represented in the public sequence databases by the accession NM_006023 and is described in this patent by Seq ID 370. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. D123 is repressed in macrophages activated by LPS and gamma interferon and is also repressed in macrophages activated by IL-15. D123 protein is an important regulator of the cell cycle [Onisto et al 1998, Exp Cell Res 242:451-9]. Recently it has been shown to be regulated by modification and turnover [Okuda et al 2001, Cell Struct Funct 26:205-14]. We have shown the hypoxia-regulation of this protein, and also of several prolyl hydroxylases which are known to target proteins for ubiquitination and proteasomal degradation. We believe that concerted hypoxic control of D123 and its regulating prolyl hydroxylase is part of the means of hypoxic regulation of cell growth and tissue re-modelling.

The Oxford BioMedica clone p1C8 represents DEC-1. The protein sequence encoded by DEC-1 is represented in the public databases by the accession NP_003661 and is described in this patent by Seq ID 371. The nucleotide sequence is represented in the public sequence databases by the accession NM_003670 and is described in this patent by Seq ID 372. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. DEC-1 is a helix-loop-helix transcription factor, and its hypoxia-regulation is highly significant. The response of renal epithelial cells to hypoxia is pertinent to kidney failure, especially regarding the medullary tissue. DEC-1 is preferentially induced by hypoxia in renal epithelial cells. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, DEC-1 is up-regulated and also down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1C9 represents RAB-8b protein. The protein sequence encoded by RAB-8b protein is represented in the public databases by the accession NP_057614 and is described in this patent by Seq ID 373. The nucleotide sequence is represented in the public sequence databases by the accession NM_016530 and is described in this patent by Seq ID 374. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. The hypoxia regulation of

this small GTP-ase, which is involved in intracellular membrane trafficking, is highly significant. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. RAB-8b protein is induced in macrophages activated by LPS and gamma interferon.

The Oxford BioMedica clone p1C10 represents Regulator of G-protein signalling 1. The protein sequence encoded by Regulator of G-protein signalling 1 is represented in the public databases by the accession NP_002913 and is described in this patent by Seq ID 375. The nucleotide sequence is represented in the public sequence databases by the accession NM_002922 and is described in this patent by Seq ID 376. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. There is a prejudice in the art that the response to hypoxia is generic to all cell types. Contrary to this, we show that genes are regulated by hypoxia to a greater degree in certain cell types, substantiating their utility in designing specific therapeutic products for diseases involving those cell types. Monocytes and macrophages have been implicated in the following diseases involving hypoxia: rheumatoid arthritis, atherosclerosis, cancer, COPD. Regulator of G-protein signalling 1 is preferentially induced by hypoxia in monocytes or macrophages. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Regulator of G-protein signalling 1 is repressed in macrophages activated by LPS and gamma interferon. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Regulator of G-protein signalling 1 is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1C11 represents Polyubiquitin. The protein sequence encoded by Polyubiquitin is represented in the public databases by the accession BAA23632 and is described in this patent by Seq ID 377. The nucleotide sequence is represented in the public sequence databases by the accession AB009010 and is described in this patent by Seq ID 378. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Polyubiquitin is repressed in macrophages activated by LPS and gamma interferon. TNFalpha is an inflammatory cytokine, which acts

on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. Polyubiquitin is induced in macrophages activated by TNFalpha. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Polyubiquitin is up-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1C12 represents Integrin, alpha 5. The protein sequence encoded by Integrin, alpha 5 is represented in the public databases by the accession NP_002196 and is described in this patent by Seq ID 379. The nucleotide sequence is represented in the public sequence databases by the accession NM_002205 and is described in this patent by Seq ID 380. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Integrin, alpha 5 may play a role in the response to neuronal injury [King et al 2001, J Neurocytol 30:243-52]. Our observation of hypoxia regulation of both COX-2 and integrin, alpha 5 supports the very recent suggestion that they may both function in recovery from cardiovascular injury [Hein et al 2001, Am J Physiol Heart Circ Physiol 281:H2378-84], which is pre-figured by our claims. Integrin, alpha 5 is induced by hypoxia in mammary epithelial cells, and may play an important role in cancer progression in that tissue through its function of regulating interaction with the extracellular matrix.

The Oxford BioMedica clone p1C13 represents Jk-recombination signal binding protein. The protein sequence encoded by Jk-recombination signal binding protein is represented in the public databases by the accession AAA60258 and is described in this patent by Seq ID 381. The nucleotide sequence is represented in the public sequence databases by the accession L07872 and is described in this patent by Seq ID 382. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Jk-recombination signal binding protein is repressed in macrophages activated by LPS and gamma interferon. TNFalpha is an inflammatory cytokine, which acts on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. Jk-recombination signal binding protein is induced in macrophages activated by TNFalpha. The important role of Jk-

recombination signal binding protein in the regulation of the immune response is thus modulated by hypoxia, and there are potentially many ways of exploiting that modulation in the design of diagnostics and therapeutics. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Jk-recombination signal binding protein is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient. It is of particular interest and significance, in view of the escape from immunological surveillance of many tumours, that Jk-recombination signal binding protein is down-regulated.

The Oxford BioMedica clone p1C14 represents Abstrakt. The protein sequence encoded by Abstrakt is represented in the public databases by the accession NP_057306 and is described in this patent by Seq ID 383. The nucleotide sequence is represented in the public sequence databases by the accession NM_016222 and is described in this patent by Seq ID 384. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Abstrakt is repressed in macrophages activated by IL-15. TNFalpha is an inflammatory cytokine, which acts on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. Abstrakt is induced in macrophages activated by TNFalpha. The general role of Abstrakt in the regulation of gene expression [Schmucker et al 2000, Mech Dev 91:189-96] implies particular significance to the recovery of cells from hypoxic insult.

The Oxford BioMedica clone p1C15 represents High-mobility group protein 2. The protein sequence encoded by High-mobility group protein 2 is represented in the public databases by the accession NP_002120 and is described in this patent by Seq ID 385. The nucleotide sequence is represented in the public sequence databases by the accession NM_002129 and is described in this patent by Seq ID 386. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1C16 represents Decidual protein induced by progesterone. The protein sequence encoded by Decidual protein induced by progesterone is represented in the public databases by the accession NP_008952 and is described in this patent by Seq ID 387. The nucleotide sequence is represented in the public sequence databases by the accession NM_007021 and is described in this patent

by Seq ID 388. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Decidual protein induced by progesterone is preferentially induced by hypoxia in mammary epithelial cells. Human decidual cells have not been tested, but we predict that Decidual protein induced by progesterone is hypoxia-regulated in those cells. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Decidual protein induced by progesterone is repressed in macrophages activated by IL-17.

The Oxford BioMedica clone p1C19 represents GM2 ganglioside activator protein. The protein sequence encoded by GM2 ganglioside activator protein is represented in the public databases by the accession NP_000396 and is described in this patent by Seq ID 389. The nucleotide sequence is represented in the public sequence databases by the accession NM_000405 and is described in this patent by Seq ID 390. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. There is a prejudice in the art that the response to hypoxia is generic to all cell types. Contrary to this, we show that genes are regulated by hypoxia to a greater degree in certain cell types, substantiating their utility in designing specific therapeutic products for diseases involving those cell types. Monocytes and macrophages have been implicated in the following diseases involving hypoxia: rheumatoid arthritis, atherosclerosis, cancer, COPD. GM2 ganglioside activator protein is preferentially induced by hypoxia in monocytes or macrophages. The hypoxia-inducibility of this protein in macrophages is likely to be clinically very significant. It is likely to play a role in the control of inflammation in asthma and inflammatory bowel disease, and in lipid metabolism and phosphatidylinositol-mediated signalling.

The Oxford BioMedica clone p1C20 represents CNOT8. The protein sequence encoded by CNOT8 is represented in the public databases by the accession NP_004770 and is described in this patent by Seq ID 391. The nucleotide sequence is represented in the public sequence databases by the accession NM_004779 and is described in this patent by Seq ID 392. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The protein sequence encoded by Similar to Nucleoside phosphorylase is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession AA430382 and is described in this patent by Seq ID 394. Hypoxia is an important

feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1P5 represents SCYA2. The protein sequence encoded by SCYA2 is represented in the public databases by the accession NP_002973 and is described in this patent by Seq ID 395. The nucleotide sequence is represented in the public sequence databases by the accession NM_002982 and is described in this patent by Seq ID 396. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. SCYA2 is induced in macrophages activated by LPS and gamma interferon and is also induced in macrophages activated by IL-17 or IL-15. Thus the role of SCYA2 in monocyte recruitment [Lu et al 1998, J Exp Med 187:601-8], which has clear relevance to the diagnosis and treatment of cardiovascular disease, cancer, rheumatoid arthritis, atherosclerosis and COPD, is enhanced by hypoxia. TNFalpha is an inflammatory cytokine, which acts on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. SCYA2 is repressed in macrophages activated by TNFalpha. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, SYCA2 is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p2L23 represents Endothelin 1. The protein sequence encoded by Endothelin 1 is represented in the public databases by the accession NP_001946 and is described in this patent by Seq ID 397. The nucleotide sequence is represented in the public sequence databases by the accession NM_001955 and is described in this patent by Seq ID 398. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Endothelin 1 is induced in macrophages activated by LPS and gamma interferon. TNFalpha is an inflammatory cytokine, which acts on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have

utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. Endothelin 1 is induced in macrophages activated by TNFalpha. Endothelin 1 plays an important role in inducing proliferation of vascular smooth muscle cells. Its hypoxia-inducibility and thus its modulation to ameliorate the consequences of ischaemic insult, is of considerable clinical significance to the recovery from injury, and angiogenesis.

The protein sequence encoded by Similar to Heat shock 70kD protein 4 is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession AA633656 and is described in this patent by Seq ID 400. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1K9 represents Lipocortin I. The protein sequence encoded by Lipocortin I is represented in the public databases by the accession NP_000691 and is described in this patent by Seq ID 401. The nucleotide sequence is represented in the public sequence databases by the accession NM_000700 and is described in this patent by Seq ID 402. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Lipocortin I (also called annexin I) is an important anti-inflammatory mediator, and its hypoxia-inducibility has important implications for the diagnosis and treatment of ischaemic disease, cancer, atherosclerosis, and inflammatory diseases such as rheumatoid arthritis. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Lipocortin I is up-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Lipocortin I is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1K23 represents MYC. The protein sequence encoded by MYC is represented in the public databases by the accession NP_002458 and is described in this patent by Seq ID 403. The nucleotide sequence is represented in the public sequence databases by the accession NM_002467 and is described in this patent by Seq ID 404. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. MYC is repressed in macrophages activated by LPS and gamma interferon. Hypoxia is frequently found in human tumours

where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, MYC is up-regulated and also down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1K15 represents Alpha-2-macroglobulin. The protein sequence encoded by Alpha-2-macroglobulin is represented in the public databases by the accession NP_000005 and is described in this patent by Seq ID 405. The nucleotide sequence is represented in the public sequence databases by the accession NM_000014 and is described in this patent by Seq ID 406. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. There is a prejudice in the art that the response to hypoxia is generic to all cell types. Contrary to this, we show that genes are regulated by hypoxia to a greater degree in certain cell types, substantiating their utility in designing specific therapeutic products for diseases involving those cell types. Monocytes and macrophages have been implicated in the following diseases involving hypoxia: rheumatoid arthritis, atherosclerosis, cancer, COPD. Alpha-2-macroglobulin is preferentially induced by hypoxia in monocytes or macrophages. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Alpha-2-macroglobulin is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1K8 represents SCYA4. The protein sequence encoded by SCYA4 is represented in the public databases by the accession XP_008449 and is described in this patent by Seq ID 407. The nucleotide sequence is represented in the public sequence databases by the accession XM_008449 and is described in this patent by Seq ID 408. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. SCYA4 is induced in macrophages activated by LPS and gamma interferon and is also induced in macrophages activated by IL-17. TNFalpha is an inflammatory cytokine, which acts on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. SCYA4 is induced in macrophages activated by TNFalpha. SCYA4 is a chemokine which is likely to be significant in inflammatory disease as a direct result of its hypoxic regulation. Hypoxia is frequently found in human tumours where macrophage

infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, SCYA4 is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1M24 represents Sex hormone-binding globulin. The protein sequence encoded by Sex hormone-binding globulin is represented in the public databases by the accession NP_001031 and is described in this patent by Seq ID 409. The nucleotide sequence is represented in the public sequence databases by the accession NM_001040 and is described in this patent by Seq ID 410. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1K7 represents ATP-binding cassette E1. The protein sequence encoded by ATP-binding cassette E1 is represented in the public databases by the accession NP_002931 and is described in this patent by Seq ID 411. The nucleotide sequence is represented in the public sequence databases by the accession NM_002940 and is described in this patent by Seq ID 412. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. ATP-binding cassette E1 is repressed in macrophages activated by LPS and gamma interferon.

The Oxford BioMedica clone p1K16 represents CCT6A. The protein sequence encoded by CCT6A is represented in the public databases by the accession NP_001753 and is described in this patent by Seq ID 413. The nucleotide sequence is represented in the public sequence databases by the accession NM_001762 and is described in this patent by Seq ID 414. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1K18 represents Colony-stimulating factor1. The protein sequence encoded by Colony-stimulating factor1 is represented in the public databases by the accession AAA52117 and is described in this patent by Seq ID 415. The nucleotide sequence is represented in the public sequence databases by the accession M37435 and is described in this patent by Seq ID 416. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease

sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Colony-stimulating factor1 is repressed in macrophages activated by LPS and gamma interferon.

The Oxford BioMedica clone p1N1 represents GA17. The protein sequence encoded by GA17 is represented in the public databases by the accession NP_006351 and is described in this patent by Seq ID 417. The nucleotide sequence is represented in the public sequence databases by the accession NM_006360 and is described in this patent by Seq ID 418. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1K22 represents GPR44. The protein sequence encoded by GPR44 is represented in the public databases by the accession NP_004769 and is described in this patent by Seq ID 419. The nucleotide sequence is represented in the public sequence databases by the accession NM_004778 and is described in this patent by Seq ID 420. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. GPR44 is repressed in macrophages activated by LPS and gamma interferon. GPR44 is most similar to the chemoattractant GPCR's [Marchese et al 1999, Genomics 1999 Feb 15;56(1):12-21]. Our demonstration of its hypoxic regulation enables prediction of roles in diseases associated with transient hypoxia and macrophages. GPCR's are a druggable class of molecules, and represent an ideal route for pharmacological intervention.

The Oxford BioMedica clone p1K14 represents Keratin 6B. The protein sequence encoded by Keratin 6B is represented in the public databases by the accession NP_005546 and is described in this patent by Seq ID 421. The nucleotide sequence is represented in the public sequence databases by the accession NM_005555 and is described in this patent by Seq ID 422. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Keratin 6B is induced in macrophages treated with the inhibitory cytokine IL-10. Keratin 6B is repressed in macrophages activated by IL-17 and is also repressed in macrophages activated by IL-15.

The Oxford BioMedica clone p1K13 represents Lymphocyte adaptor protein. The protein sequence encoded by Lymphocyte adaptor protein is represented in the public databases by the accession NP_005466 and is described in this patent by Seq ID 423. The nucleotide sequence is represented in the

public sequence databases by the accession NM_005475 and is described in this patent by Seq ID 424. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1J20 represents Neuro-oncological ventral antigen 1. The protein sequence encoded by Neuro-oncological ventral antigen 1 is represented in the public databases by the accession NP_002506 and is described in this patent by Seq ID 425. The nucleotide sequence is represented in the public sequence databases by the accession NM_002515 and is described in this patent by Seq ID 426. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Neuro-oncological ventral antigen 1 is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1J22 represents Neutral sphingomyelinase (N-SMase) activation associated factor. The protein sequence encoded by Neutral sphingomyelinase (N-SMase) activation associated factor is represented in the public databases by the accession NP_003571 and is described in this patent by Seq ID 427. The nucleotide sequence is represented in the public sequence databases by the accession NM_003580 and is described in this patent by Seq ID 428. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Neutral sphingomyelinase (N-SMase) activation associated factor is induced in macrophages treated with the inhibitory cytokine IL-10. Neutral sphingomyelinase (N-SMase) activation associated factor is repressed in macrophages activated by IL-17 and is also repressed in macrophages activated by IL-15. We expect activation of of Neutral sphingomyelinase (N-SMase) to have an anti-inflammatory effect. This enzyme is known to modulate the sphingomyelin second messenger cycle, potentially interacting with the oxidative system. Our demonstration of hypoxic regulation provides a crucial indication of the benefit of therapeutic intervention via sphingomyelinase (N-SMase) for the treatment of inflammatory diseases and diseases related to the hypoxic macrophage.

The Oxford BioMedica clone p1K1 represents Cyclophilin F. The protein sequence encoded by Cyclophilin F is represented in the public databases by the accession NP_005720 and is described in this patent by Seq ID 429. The nucleotide sequence is represented in the public sequence databases by the accession NM_005729 and is described in this patent by Seq ID 430. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Hypoxia is frequently found

in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Cyclophilin F is up-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1K3 represents Pleckstrin. The protein sequence encoded by Pleckstrin is represented in the public databases by the accession NP_002655 and is described in this patent by Seq ID 431. The nucleotide sequence is represented in the public sequence databases by the accession NM_002664 and is described in this patent by Seq ID 432. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. There is a prejudice in the art that the response to hypoxia is generic to all cell types. Contrary to this, we show that genes are regulated by hypoxia to a greater degree in certain cell types, substantiating their utility in designing specific therapeutic products for diseases involving those cell types. Monocytes and macrophages have been implicated in the following diseases involving hypoxia: rheumatoid arthritis, atherosclerosis, cancer, COPD. Pleckstrin is preferentially induced by hypoxia in monocytes or macrophages. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Pleckstrin is induced in macrophages activated by LPS and gamma interferon. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Pleckstrin is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clones p1J19 and p1K2 represent CFFM4. The protein sequence encoded by CFFM4 is represented in the public databases by the accession NP_067024 and is described in this patent by Seq ID 433. The nucleotide sequence is represented in the public sequence databases by the accession NM_021201 and is described in this patent by Seq ID 434. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. There is a prejudice in the art that the response to hypoxia is generic to all cell types. Contrary to this, we show that genes are regulated by hypoxia to a greater degree in certain cell types, substantiating their utility in designing specific therapeutic products for diseases involving those cell types. Monocytes and macrophages have been implicated in the following diseases involving hypoxia: rheumatoid arthritis, atherosclerosis, cancer, COPD. CFFM4 is preferentially induced by hypoxia in monocytes or macrophages. CFFM4 is induced in macrophages treated with the inhibitory cytokine IL-10. It has been suggested recently that CFFM4 is associated with mature cellular function in the monocytic lineage and that it may be a component of a

receptor complex involved in signal transduction [Gingras et al 2001, Immunogenetics 53:468-76]. Our demonstration of hypoxic-regulation opens possible routes of intervention in macrophage-related disease via this potentially important cell surface receptor. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, CFFM4 is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1K5 represents Ribosomal protein L36a. The protein sequence encoded by Ribosomal protein L36a is represented in the public databases by the accession NP_000992 and is described in this patent by Seq ID 435. The nucleotide sequence is represented in the public sequence databases by the accession NM_001001 and is described in this patent by Seq ID 436. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1J17 represents SLC6A1. The protein sequence encoded by SLC6A1 is represented in the public databases by the accession NP_003033 and is described in this patent by Seq ID 437. The nucleotide sequence is represented in the public sequence databases by the accession NM_003042 and is described in this patent by Seq ID 438. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, SLC6A1 is up-regulated and also down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1J18 represents Synaptopodin. The protein sequence encoded by Synaptopodin is represented in the public databases by the accession NP_009217 and is described in this patent by Seq ID 439. The nucleotide sequence is represented in the public sequence databases by the accession NM_007286 and is described in this patent by Seq ID 440. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Synaptopodin is a component of the cytoskeleton which has particular importance in neurons, where it is involved in synaptic plasticity. Its hypoxia-regulation is clearly potentially significant in the context of neurological disease. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Synaptopodin is up-regulated and also down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1J15 represents TERA protein. The protein sequence encoded by TERA protein is represented in the public databases by the accession NP_067061 and is described in this patent by Seq ID 441. The nucleotide sequence is represented in the public sequence databases by the accession NM_021238 and is described in this patent by Seq ID 442. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, TERA protein is up-regulated and also down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1K4 represents TSC-22. The protein sequence encoded by TSC-22 is represented in the public databases by the accession NP_006013 and is described in this patent by Seq ID 443. The nucleotide sequence is represented in the public sequence databases by the accession NM_006022 and is described in this patent by Seq ID 444. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. TSC-22 is a transcriptional regulator of the leucine zipper class, and its hypoxic regulation is likely to have significant downstream effects which may be related to ischaemic disease. Thus it may provide important points of intervention in such diseases. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. TSC-22 is repressed in macrophages activated by LPS and gamma interferon. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, TSC-22 is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p2A14 represents an unannotated EST. The protein sequence encoded by this EST is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession AA988110 and is described in this patent by Seq ID 446. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. The EST represented by Seq ID 446 is induced in macrophages treated with the inhibitory cytokine IL-10. The EST represented by Seq ID 446 is repressed in macrophages activated by IL-17 and is also repressed in macrophages activated by IL-15.

The Oxford BioMedica clone p1J23 represents Calgranulin A. The protein sequence encoded by Calgranulin A is represented in the public databases by the accession NP_002955 and is described in this

patent by Seq ID 447. The nucleotide sequence is represented in the public sequence databases by the accession NM_002964 and is described in this patent by Seq ID 448. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Calgranulin A, called by its synonym S100A8, has been cited recently as "wound-regulated" [Thorey et al 2001, J Biol Chem 276:35818-25] which provides less precise support for our prior determination of its hypoxia-regulation. In its potential role as a chemoattractant, it would be an important point of intervention for the modulation of inflammatory processes. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Calgranulin A is repressed in macrophages activated by LPS and gamma interferon. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Calgranulin A is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1J21 represents Replication factor C large subunit. The protein sequence encoded by Replication factor C large subunit is represented in the public databases by the accession NP_002904 and is described in this patent by Seq ID 449. The nucleotide sequence is represented in the public sequence databases by the accession NM_002913 and is described in this patent by Seq ID 450. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1J24 represents Signal recognition particle 19kD. The protein sequence encoded by Signal recognition particle 19kD is represented in the public databases by the accession NP_003126 and is described in this patent by Seq ID 451. The nucleotide sequence is represented in the public sequence databases by the accession NM_003135 and is described in this patent by Seq ID 452. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1J16 represents cDNA: FLJ23019 fis, clone LNG00916. The protein sequence encoded by cDNA: FLJ23019 fis, clone LNG00916 is not represented in the public databases by a protein accession. The nucleotide sequence is represented in the public sequence databases by the accession AK026672 and is described in this patent by Seq ID 454. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to

several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. The cDNA: FLJ23019 fis, clone LNG00916 is induced in macrophages activated by LPS and gamma interferon and is also induced in macrophages activated by IL-15.

The Oxford BioMedica clone p1J2 represents Proteasome subunit, alpha type, 4. The protein sequence encoded by Proteasome subunit, alpha type, 4 is represented in the public databases by the accession NP_002780 and is described in this patent by Seq ID 455. The nucleotide sequence is represented in the public sequence databases by the accession NM_002789 and is described in this patent by Seq ID 456. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1J9 represents MAFB. The protein sequence encoded by MAFB is represented in the public databases by the accession NP_005452 and is described in this patent by Seq ID 457. The nucleotide sequence is represented in the public sequence databases by the accession NM_005461 and is described in this patent by Seq ID 458. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. MAFB is a transcriptional regulator of the leucine zipper type, and is likely to play an important role in the mediation of the hypoxic response, with attendant relevance to associated diseases.

The Oxford BioMedica clone p1J10 represents DNCLI2. The protein sequence encoded by DNCLI2 is represented in the public databases by the accession NP_006132 and is described in this patent by Seq ID 459. The nucleotide sequence is represented in the public sequence databases by the accession NM_006141 and is described in this patent by Seq ID 460. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, gene X is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1J1 represents Chromobox homolog 3. The protein sequence encoded by Chromobox homolog 3 is represented in the public databases by the accession NP_057671 and is described in this patent by Seq ID 461. The nucleotide sequence is represented in the public sequence databases by the accession NM_016587 and is described in this patent by Seq ID 462. Hypoxia is an

important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1J5 represents SCYA7. The protein sequence encoded by SCYA7 is represented in the public databases by the accession NP_006264 and is described in this patent by Seq ID 463. The nucleotide sequence is represented in the public sequence databases by the accession NM_006273 and is described in this patent by Seq ID 464. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. SCYA7 is induced in macrophages activated by IL-15. SCYA7 is a chemoattractant protein which, considering its hypoxia-regulation, is likely to play an important role in inflammatory and ischaemic disease. TNFalpha is an inflammatory cytokine, which acts on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. SCYA7 is repressed in macrophages activated by TNFalpha.

The Oxford BioMedica clone p1J11 represents Fatty-acid-Coenzyme A ligase, long-chain 2. The protein sequence encoded by Fatty-acid-Coenzyme A ligase, long-chain 2 is represented in the public databases by the accession NP_066945 and is described in this patent by Seq ID 465. The nucleotide sequence is represented in the public sequence databases by the accession NM_021122 and is described in this patent by Seq ID 466. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Fatty-acid-Coenzyme A ligase, long-chain 2 is induced in macrophages activated by LPS and gamma interferon and also induced in macrophages activated by IL-17 or IL-15. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Fatty-acid-Coenzyme A ligase, long-chain 2 is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1J8 represents Programmed cell death 5. The protein sequence encoded by Programmed cell death 5 is represented in the public databases by the accession NP_004699 and is

described in this patent by Seq ID 467. The nucleotide sequence is represented in the public sequence databases by the accession NM_004708 and is described in this patent by Seq ID 468. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1I20 represents SCYA3L. The protein sequence encoded by SCYA3L is represented in the public databases by the accession CAA36397 and is described in this patent by Seq ID 469. The nucleotide sequence is represented in the public sequence databases by the accession X52149 and is described in this patent by Seq ID 470. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. There is a prejudice in the art that the response to hypoxia is generic to all cell types. Contrary to this, we show that genes are regulated by hypoxia to a greater degree in certain cell types, substantiating their utility in designing specific therapeutic products for diseases involving those cell types. Monocytes and macrophages have been implicated in the following diseases involving hypoxia: rheumatoid arthritis, atherosclerosis, cancer, COPD. SCYA3L is preferentially induced by hypoxia in monocytes or macrophages. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. SCYA3L is induced in macrophages activated by LPS and gamma interferon. TNFalpha is an inflammatory cytokine, which acts on macrophages, and has been shown to be central to the pathophysiology and treatment of diseases including rheumatoid arthritis. Genes that change in expression in response to TNFalpha therefore have utility in the design of therapeutic, prognostic and diagnostic products for such inflammatory conditions. SCYA3L is induced in macrophages activated by TNFalpha.

The Oxford BioMedica clone p1J3 represents Furin. The protein sequence encoded by Furin is represented in the public databases by the accession NP_002560 and is described in this patent by Seq ID 471. The nucleotide sequence is represented in the public sequence databases by the accession NM_002569 and is described in this patent by Seq ID 472. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1J12 represents Nuclear autoantigenic sperm protein. The protein sequence encoded by Nuclear autoantigenic sperm protein is represented in the public databases by the accession NP_002473 and is described in this patent by Seq ID 473. The nucleotide sequence is represented in the public sequence databases by the accession NM_002482 and is described in this patent by Seq ID 474. Hypoxia is an important feature of several diseases, and genes that respond to this

stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1I23 represents Ecotropic viral integration site 2A. The protein sequence encoded by Ecotropic viral integration site 2A is represented in the public databases by the accession NP_055025 and is described in this patent by Seq ID 475. The nucleotide sequence is represented in the public sequence databases by the accession NM_014210 and is described in this patent by Seq ID 476. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. There is a prejudice in the art that the response to hypoxia is generic to all cell types. Contrary to this, we show that genes are regulated by hypoxia to a greater degree in certain cell types, substantiating their utility in designing specific therapeutic products for diseases involving those cell types. Monocytes and macrophages have been implicated in the following diseases involving hypoxia: rheumatoid arthritis, atherosclerosis, cancer, COPD. Ecotropic viral integration site 2A is preferentially induced by hypoxia in monocytes or macrophages. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Ecotropic viral integration site 2A is repressed in macrophages activated by LPS and gamma interferon. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Ecotropic viral integration site 2A is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1J7 represents Sjogren syndrome antigen B. The protein sequence encoded by Sjogren syndrome antigen B is represented in the public databases by the accession NP_003133 and is described in this patent by Seq ID 477. The nucleotide sequence is represented in the public sequence databases by the accession NM_003142 and is described in this patent by Seq ID 478. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Sjogren syndrome antigen B is preferentially induced by hypoxia in mammary epithelial cells. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. Sjogren syndrome antigen B is induced in macrophages activated by LPS and gamma interferon.

The Oxford BioMedica clone p1I21 represents SCYA8. The protein sequence encoded by SCYA8 is represented in the public databases by the accession NP_005614 and is described in this patent by Seq ID

479. The nucleotide sequence is represented in the public sequence databases by the accession NM_005623 and is described in this patent by Seq ID 480. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. SCYA8 is induced in macrophages activated by LPS and gamma interferon and is also induced in macrophages activated by IL-15.

The Oxford BioMedica clone p1I19 represents GRO2. The protein sequence encoded by GRO2 is represented in the public databases by the accession NP_002080 and is described in this patent by Seq ID 481. The nucleotide sequence is represented in the public sequence databases by the accession NM_002089 and is described in this patent by Seq ID 482. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. GRO2 is induced in macrophages activated by LPS and gamma interferon and is also induced in macrophages activated by IL-17. GRO2 encodes a chemokine which is likely to be involved in the inflammatory response. Its induction by hypoxia provides a potential route for intervention in diseases related to inflammation and ischaemia. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, GRO2 is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1J4 represents Small nuclear ribonucleoprotein D1. The protein sequence encoded by Small nuclear ribonucleoprotein D1 is represented in the public databases by the accession NP_008869 and is described in this patent by Seq ID 483. The nucleotide sequence is represented in the public sequence databases by the accession NM_006938 and is described in this patent by Seq ID 484. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products.

The Oxford BioMedica clone p1I24 represents GRO1. The protein sequence encoded by GRO1 is represented in the public databases by the accession NP_001502 and is described in this patent by Seq ID 485. The nucleotide sequence is represented in the public sequence databases by the accession

NM_001511 and is described in this patent by Seq ID 486. GRO1 has known chemotactic activity for neutrophils. GRO1 belongs to the intercrine alpha family of small CXC cytokines. GRO1 encodes a chemokine which is likely to be involved in the inflammatory response. Its induction by hypoxia provides a potential route for intervention in diseases related to inflammation and ischaemia. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Macrophages are key to several diseases involving hypoxia, and contribute to inflammatory processes. In these, macrophages are frequently activated by cytokines, which have been shown to be present at disease sites, so gene expression responses to both hypoxia and cytokines are especially relevant. GRO1 is induced in macrophages activated by LPS and gamma interferon and is also induced in macrophages activated by IL-17. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, GRO1 is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

The Oxford BioMedica clone p1I18 represents Selectin L. The protein sequence encoded by Selectin L is represented in the public databases by the accession NP_000646 and is described in this patent by Seq ID 487. The nucleotide sequence is represented in the public sequence databases by the accession NM_000655 and is described in this patent by Seq ID 488. Hypoxia is an important feature of several diseases, and genes that respond to this stimulus are therefore implicated in the pathogenesis and have utility in the design of therapeutic, prognostic and diagnostic products. Selectin L shedding by leucocytes is one aspect of the induction of the inflammatory response. Hypoxic-regulation of Selectin L is clearly a significant factor in the induction of inflammation following ischaemic insult or in diseases in which transient ischaemic conditions occur. Modulation of this induction is one aspect of the present invention. Hypoxia is frequently found in human tumours where macrophage infiltrates are also found. In a series of 5 patients with either ovarian or breast cancer, Selectin L is down-regulated in the malignant tissue as compared to adjacent normal tissue in at least one patient.

TABLES

TABLE 1: Hypoxia-inducible genes identified from clones **only** derived from the cardiomyoblast library

	SEQ ID		
GENE NAME	protein	nucleotide	**Accession**
Diacylglycerol kinase, zeta	353	354	NM_003646
CCR4 associated factor 1	391	392	AF053318
GM2 ganglioside activator protein.	389	390	X62078
Granulin	269	270	AK000607
Serine protease 11	355	356	Y07921
High mobility group 2 protein	385	386	M83665
Decidual protein induced by progesterone	387	388	NM_007021
DEAD-box protein abstrakt	383	384	NM_016222
IL-1 receptor antagonist	357	358	U65590
KIAA1376 protein	29	30	AB037797
Hypothetical protein KIAA0127	31	32	D50917
Hypothetical protein FLJ20308	33	34	AL137263
EST	91	92	AL390082
EST	89	90	AL117352
EST	77	78	AW664180

TABLE 2: Hypoxia-inducible genes identified from clones only derived from the macrophage libraries

	SEQ ID		
GENE NAME	protein	nucleotide	Accession
Metallothionein-2a	265	266	J00271
Metallothionein-1h	239	240	X64177
Metallothionein-1G	243	244	J03910
Interleukin 8	251	252	Y00787
Lactate dehydrogenase A	223	224	NM_005566
UDP-glucose pyrophosphorylase 2	347	348	NM_006759
Enolase 1	257	258	NM_001428
Enolase 2	273	274	NM_001975
Tissue factor / coagulation factor III / thromboplastin	225	226	NM_001993
proline 4-hydroxylase, alpha polypeptide 1	231	232	NM_000917
proline 4-hydroxylase, alpha polypeptide II	349	350	NM_004199
NS1-binding protein	359	360	NM_006469
FGF receptor activating protein 1	363	364	AF159621
Adenylate kinase 3	263	264	NM_013410
Osteopontin	267	268	X13694
Aldolase C, fructose-bisphosphate	259	260	NM_005165
Galectin-8	365	366	AF193806
Regulator of G-protein signalling 1 (BL34)	375	376	S59049
Polyubiquitin UbC	377	378	AB009010
Activin A receptor type I	361	362	NM_001105
Glyceraldehyde-3-phosphate dehydrogenase	253	254	NM_002046
Phosphoglycerate kinase 1	255	256	NM_000291
Rab-8b	373	374	NM_016530
Glucose phosphate isomerase	367	368	NM_000175
D123 gene product (HT1080)	369	370	U27112
Integrin alpha 5	379	380	NM_002205
Triosephosphate isomerase 1	261	262	NM_000365
solute carrier family 31 (copper transporters),	345	346	NM_001860

member 2			
Jk-recombination signal binding protein	381	382	L07872
N-myc downstream regulated (NDRG1/ RTP)	229	230	D87953
Plasminogen activator inhibitor-1	235	236	M16006
Dec-1	371	372	NM_003670
FUSIN / CXCR4	331	332	NM_003467
Hypothetical protein FLJ20500	25	26	AK000507
DKFZP564D116 protein	27	28	AL050022
Hypothetical protein FLJ10134	23	24	AK000996
cDNA FLJ10433 fis NT2RP1000478	73	74	AK001295
ESTs	93	94	AW250104
ESTs	95	96	BE382614
ESTs	67	68	AW071063
ESTs	67	68	AW964331
ESTs	133	134	AA612751
Singleton EST (not in UniGene)	135	136	AI018611

The gene entitled "Jk-recombination signal binding protein" was found to be hypoxia-inducible using subtracted cDNA probes for hybridization, but with non-subtracted probes, where the hybridisation is quantitative, no signal was detected. This indicates that the gene is probably hypoxia-regulated but the absolute expression levels are very low.

TABLE 3: Hypoxia-inducible genes identified from clones derived from **both** macrophage and myoblast libraries.

		SEQ ID		Hypoxia/	Hypoxia/
GENE NAME	**Accession**	protein	nucleotide	**normoxia**	**normoxia**
				(macrophage)	**(myoblast)**
Solute carrier family 2, member 3	NM_006931	247	248	91.39	8.23
Solute carrier family 2, member 5	NM_003039	311	312	10.75	2.26
Adiphophilin	NM_001122	313	314	13.97	5.10
Hexokinase 2	NM_000189	249	250	11.50	6.25
Stearoyl-CoA desaturase	AB032261	351	352	3.74	2.31
cDNA DKFZp434O071	AF125392	75	76	2.31	2.75
Hypoxia-inducible protein 2	NM_013332	271	272	3.62	5.07

TABLE 4: Hypoxia responses amplified by HIF1alpha overexpression

Gene Name	Nucl	Experimental Condition #								
	Seq ID	1	2	3	4	5	6	7	8	9
Metallothionein 2A	265	1	0.57	0.69	3.33	3.22	5.77	10.37	2.05	1.70
Metallothionein 1G	244	1	0.68	0.64	4.23	4.21	7.35	11.03	3.65	2.28
Hypothetical protein hqp0376	338	1	0.79	0.61	6.54	4.44	9.01	11.54	4.17	3.22
Novel Metallothionein	84	1	0.95	0.78	5.18	4.36	8.20	11.16	3.48	2.94

Legend: Data shown in the average of 4 repeat experiments. Experimental condition is as shown in the text. Values represent fold change as compared to untreated cells (condition 1).

TABLE 5: Hypoxia responses amplified by EPAS1 overexpression

Gene Name	Nucl Seq ID	Experimental Condition #								
		1	2	3	4	5	6	7	8	9
cDNA DKFZp586E1624	66	1	0.77	0.67	1.00	1.12	1.58	0.83	2.60	2.49
Butyrate response factor 1	328	1	0.74	0.64	1.60	1.64	1.57	1.23	2.19	3.20
hypothetical protein FLJ10134	24	1	0.62	0.53	2.73	2.09	2.80	2.87	4.20	3.65
EGL nine (C.elegans) homolog 3	86	1	1.34	0.81	1.98	1.90	2.02	1.94	2.81	3.12
ERO1 (S. cerevisiae)-like	68	1	1.02	1.30	4.26	4.14	4.76	4.12	4.91	6.44
hypothetical protein FLJ10134	24	1	0.68	0.53	2.03	1.97	3.01	2.46	3.67	2.95

Legend: Data shown is the average of 4 repeat experiments. Experimental condition is as shown in the text. Values represent fold change as compared to untreated cells (condition 1).

TABLE 6. Negative hypoxia responses amplified by HIF1alpha / EPAS1 overexpression

Gene Name	Nucl Seq ID	Experimental Condition #								
		1	2	3	4	5	6	7	8	9
Hypothetical protein CGI-117	48	1	0.83	0.87	0.42	0.42	0.32	0.34	0.33	0.27

Table 7: Genes induced by hypoxia (similar response +/- cell activation)

						RATIO		
				SEQ ID		Hypoxia / Normoxia		Activated / Resting
Row	TITLE	IMAGE Id	accession	protein	nucl	(resting)	(activated)	(normoxia)
1	Activated leucocyte cell adhesion molecule	26617	R13558	277	278	1.46	1.86	0.46
2	MAX-interacting protein 1	435219	AA705886	279	280	2.55	3.18	n/d
3	BCL2/adenovirus E1B 19kD-interacting protein 3-like	814899	AA465697	217	218	2.50	3.48	0.41
4	Nuclear receptor co-repressor	488301	AA085748	281	282	1.38	1.75	0.65
5	Enolase 2, (gamma, neuronal)	789147	AA450189	273	274	2.87	4.98	1.32
6	Chitinase 3-like 2	47043	H10721	283	284	1.98	1.98	n/d
7	BACH1 transcription factor	2009495	AI336948	285	286	2.34	2.23	1.30
8	Solute carrier family 2, member 1	453589	AA679565	219	220	8.50	6.80	0.59
9	Phosphoglucomutase 1	843174	AA488504	287	288	1.43	1.83	n/d
10	PDGF beta	67654	T49539	221	222	1.66	1.64	1.09
11	PDGF beta	343320	W68169	221	222	1.86	1.67	0.84
12	CGI-109 protein	144862	R78570	289	290	1.42	1.94	n/d
13	SAP30	502142	AA126982	291	292	2.03	3.49	n/d
14	ATP-binding cassette transporter-1	827168	AA521292	293	294	2.04	2.24	1.20
15	SEC24 protein	712559	AA278134	295	296	2.87	3.97	n/d
16	Trinucleotide repeat containing 3	199367	R95691	297	298	1.92	1.38	1.35
17	Post-synaptic density protein 95	26021	R39954	299	300	1.79	1.64	1.63
18	Tumor protein D52	814306	AA459318	301	302	1.24	1.75	n/d
19	Cyclin-dependent kinase inhibitor p27kip1	854668	AA630082	303	304	2.36	1.57	2.19
20	phosphoinositide-3-kinase, catalytic, beta	506009	AA708437	305	306	1.44	2.11	0.26
21	cDNA FLJ13611 fis, clone PLACE1010802	49918	H15296	1	2	2.33	2.54	n/d

22	Solute carrier family 5, member 3	345743	W72666	307	308	3.33	4.42	1.14
23	PSCDBP	824531	AA490903	309	310	2.02	1.90	n/d
24	lactate dehydrogenase A	43550	H05914	223	224	2.13	2.23	1.23
25	Solute carrier family 2, member 5	190732	H38650	311	312	2.72	3.45	n/d
26	Adipophilin	435036	AA700054	313	314	6.28	2.39	n/d
27	Tissue factor	1928791	AI313387	225	226	1.34	2.21	0.62
28	Vascular endothelial growth factor	34778	R19956	227	228	1.53	1.97	n/d
29	RTP / NDRG1	842863	AA489261	229	230	3.40	3.06	2.38
30	Early development regulator 2	898328	AA598840	315	316	1.96	1.61	1.18
31	Procollagen-proline 4-hydroxylase alpha 1	838802	AA457671	231	232	2.69	2.32	1.31
32	B-cell translocation gene 1,	298268	N70463	317	318	1.91	2.08	1.84
33	SH3PX1	142139	R69163	319	320	1.81	1.15	1.87
34	Cyclin G2	823691	AA489752	321	322	1.70	2.47	n/d
35	BCL2/adenovirus E1B-interacting protein 3	783697	AA446839	233	234	4.37	6.52	n/d
36	BCL2/adenovirus E1B-interacting protein 3	359982	AA063521	233	234	3.09	5.00	n/d
37	NAG-5 protein	460618	AA700447	323	324	1.91	n/d	n/d
38	Cytochrome P450 IB1 (dioxin-inducible)	782760	AA448157	325	326	1.93	2.25	0.93
39	Plasminogen activator inhibitor, type I	244307	N75719	235	236	1.81	2.78	n/d
40	Butyrate response factor 1	768299	AA424743	327	328	2.59	2.69	1.88
41	Butyrate response factor 1	413633	AA723035	327	328	2.35	2.36	1.76
42	p8 protein (candidate of metastasis 1)	80484	T64469	329	330	4.13	4.19	n/d
43	Fusin / CXCR4	79629	T62491	331	332	2.16	1.97	1.07
44	solute carrier family 16, member 6	1638893	AI016779	333	334	1.96	n/d	n/d
45	solute carrier family 16, member 6	266389	N21654	333	334	2.00	n/d	n/d
46	Proline-rich protein with nuclear targeting signal (B4-2)	857002	AA669637	335	336	2.707	1.80	7.08

47	Cox-2	845477	AA644211	237	238	n/d	8.34	22.38
48	Glycogen synthase 1 (muscle)	45632	H08446	275	276	n/d	2.28	1.15
49	cDNA FLJ13700 fis, clone PLACE2000216, highly similar to SPECTRIN BETA CHAIN, BRAIN	261246	H98241	15	16	1.25	2.01	0.25
50	Hypothetical protein FLJ20037	142944	R71124	3	4	2.10	1.74	n/d
51	Hypothetical protein FLJ20037	451087	AA704517	3	4	2.36	1.73	n/d
52	hypothetical protein DKFZp434P0116	417863	W88781	5	6	2.18	1.85	0.95
53	KIAA0212	854874	AA630346	7	8	1.77	1.84	0.84
54	KIAA0914	283301	N51424	9	10	1.93	1.40	n/d
55	Hypothetical protein FLJ20281	244686	N54297	11	12	2.13	2.05	n/d
56	KIAA0876	809806	AA454753	13	14	2.80	1.82	n/d
57	DKFZP586G1122 protein	950778	AA608636	17	18	1.76	1.99	0.44
58	Putative zinc finger protein LOC55818	377452	AA055692	19	20	2.16	n/d	n/d
59	hypothetical protein PRO0823	194965	R88734	21	22	1.89	1.47	1.02
60	Hypothetical protein PRO0823	1486194	AA936866	21	22	2.17	1.31	0.46
61	cDNA DKFZp586H0324 clone DKFZp586H0324	130276	R21170	61	62	2.07	2.47	n/d
62	Clone 23785	376476	AA041362	63	64	2.50	2.29	n/d
63	Clone 23785	261834	H98855	63	64	2.14	1.97	0.49
64	cDNA DKFZp586E1624	284497	N52362	65	66	1.95	1.09	n/d
65	ESTs (UniGene annotated)	139558	R62339	79	80	1.73	1.75	1.69
66	ESTs (UniGene annotated)	897446	AA489477	81	82	1.74	1.83	n/d
67	ESTs (UniGene annotated)	126458	R06601	83	84	2.53	1.92	2.07
68	ESTs(UniGene annotated)	122982	R00332	85	86	2.43	3.96	n/d
69	ESTs (UniGene annotated)	811808	AA463469	87	88	1.79	1.31	1.24

70	ESTs(UniGene annotated)	203544	H56028	89	90	3.67	3.63	n/d
71	ESTs (UniGene annotated)	714437	AA293300	91	92	2.46	1.85	3.47
72	ESTs	810448	AA457116	67	68	4.87	2.97	1.22
73	ESTs	207275	H59618	97	98	2.61	1.24	1.04
74	ESTs	785928	AA449703	99	100	1.45	1.84	0.57
75	ESTs	827204	AA521311	101	102	1.80	1.55	1.27
76	ESTs	343695	W69170	103	104	1.78	1.48	1.69
77	ESTs	39145	R51835	105	106	1.49	1.72	1.04
78	ESTs	220608	H87770	107	108	1.47	2.09	1.09
79	ESTs	142087	R69248	109	110	1.57	1.70	n/d
80	ESTs	82171	T68844	111	112	2.44	2.16	1.19
81	ESTs	795325	AA454177	113	114	1.75	1.28	n/d
82	ESTs	366966	AA026562	115	116	1.27	1.70	n/d
83	ESTs	84419	T73780	117	118	1.43	2.22	1.07
84	ESTs	742611	AA401496	119	120	3.63	3.75	n/d
85	ESTs	277611	N49384	119	120	4.52	2.87	n/d
86	ESTs	823688	AA489636	121	122	2.11	n/d	n/d
87	ESTs	781311	AA446361	123	124	1.66	2.43	n/d
88	ESTs	1555201	AA931411	125	126	1.89	n/d	n/d
89	ESTs	131563	R24223	127	128	2.26	n/d	n/d
90	EST (singleton)	786657	AA451886	137	138	2.44	2.01	n/d
91	ESTs (ex-UniGene)	126393	R06520	139	140	1.59	1.81	0.86
92	ESTs(ex UniGene)	74054	T48278	141	142	1.92	1.05	n/d

Legend

The last 3 columns show mRNA expression as a ratio between the conditions being compared. Of these three columns the first two show expression in hypoxia relative to normoxia, done separately in resting macrophages or activated macrophages. The final column shows expression in activated macrophages relative to resting macrophages (both in normoxia) as a ratio. n/d = not determined due to low signal intensities. IMAGE ID and accession describe the exact identity of the arrayed clones and do not describe full length cDNA sequence database entries.

TABLE 8: Genes induced by hypoxia (greater response in resting cells)

						RATIO		
				SEQ ID		Hypoxia / Normoxia		Activated / Resting
Row	TITLE	IMAGE ID	accession	protein	nucl	(resting)	(activated)	(normoxia)
1	Metallothionein 1H	214162	H77766	239	240	6.26	2.01	17.58
2	Metallothionein 1L	297392	N80129	241	242	18.55	2.21	7.57
3	metallothionein 1L	1899230	AI289110	241	242	5.89	1.70	9.63
4	Metallothionein-IG	202535	H53340	243	244	12.07	2.36	21.28
5	Metallothionein 1E (functional)	1472735	AA872383	245	246	10.16	2.04	4.66
6	RNAhelicase-related protein/ metallotheionein1F	245990	N55459	337	338	6.41	1.99	14.16
7	RNAhelicase-related protein/ metallotheionein1F	78353	T56281	337	338	5.19	1.54	12.00
8	Solute carrier family 2,member 3	753467	AA406551	247	248	7.67	4.69	4.78
9	Hexokinase 2	1637282	AI005515	249	250	7.32	3.27	0.62
10	DKFZp434E1723 clone DKFZp434E1723	1593887	AA987423	69	70	2.04	1.34	1.49
11	CytochromeP450,subfamilyXXVIIB, polypeptl	1761925	AI222585	339	340	2.16	0.72	2.92
12	Interleukin 8	549933	AA102526	251	252	5.65	0.86	382.80
13	SHB adaptor protein	768362	AA495786	341	342	1.87	0.84	0.39
14	ESTs	130835	R22252	129	130	1.97	0.93	0.83

Legend to Table 8

The last 3 columns show mRNA expression as a ratio between the conditions being compared. Of these three columns the first two show expression in hypoxia relative to normoxia, done separately in resting macrophages or activated macrophages. The final column shows expression in activated macrophages relative to resting macrophages (both in normoxia) as a ratio. n/d = not determined due to low signal intensities. IMAGE ID and accession describe the exact identity of the arrayed clones and do not describe full length cDNA sequence database entries.

TABLE 9: Genes induced by hypoxia (greater response in activated cells)

					RATIO		
			SEQ ID		Hypoxia / Normoxia		Activated/ Resting
TITLE	IMAGE ID	accession	protein	nucleotide	(resting)	(activated)	(normoxia)
Papillomavirus regulatory factor (PRF-1)	744983	AA625924	343	344	3.36	8.10	0.22
cDNA FLJ11041 fis, clone PLACE1004405	140301	R66924	71	72	1.46	3.19	2.50
ESTs (ex-UniGene)	139250	R68736	143	144	1.01	2.18	1.68

Legend

The last 3 columns show mRNA expression as a ratio between the conditions being compared. Of these three columns the first two show expression in hypoxia relative to normoxia, done separately in resting macrophages or activated macrophages. The final column shows expression in activated macrophages relative to resting macrophages (both in normoxia) as a ratio. n/d = not determined due to low signal intensities. IMAGE ID and accession describe the exact identity of the arrayed clones and do not describe full length cDNA sequence database entries.

TABLE 10: Genes repressed by hypoxia (greater response in activated cells)

						RATIO		
				SEQ ID		Hypoxia / Normoxia		Activated/Resting
row	TITLE	IMAGE ID	accession	protein	nucl	(resting)	(activated)	(normoxia)
1	Maf-related leucine zipper homolog	77193	T50121	457	458	1.18	0.48	2.39
2	Alpha-2-macroglobulin	44180	H06516	405	406	1.11	0.54	1.98
3	KIAA0014	725927	AA292382	51	52	1.10	0.65	25.00
4	ESTs	178805	H49601	203	204	1.04	0.49	1.62
5	dynein, cytoplasmic, light intermediate polypeptide 2	811870	AA454959	459	460	1.03	0.42	3.31
6	Heterochromatin-like protein 1	343490	W69106	461	462	1.01	0.60	3.21
7	Monocyte chemotactic protein 3	485989	AA040170	463	464	0.89	0.52	59.62
8	Fatty-acid-Coenzyme A ligase, long-chain 2	82734	T73556	465	466	0.88	0.52	6.85
9	Fatty-acid-Coenzyme A ligase, long-chain 2	2014138	AI361530	465	466	0.72	0.46	3.97
10	Programmed cell death 5 / TFAR19	502369	AA156940	467	468	0.78	0.59	1.57
11	cDNA FLJ14028 fis, clone HEMBA1003838	366156	AA062814	145	146	0.75	0.55	1.74
12	Small inducible cytokine A3	153355	R47893	469	470	0.69	0.29	8.74
13	Cytochrome c oxidase subunit VIc	42993	R59927	471	472	0.72	0.54	1.98
14	NASP histone-binding prot.	845415	AA644128	473	474	0.64	0.38	1.64
15	Hypothetical protein HSPC196	144902	R78498	53	54	0.63	0.48	1.62
16	Ecotropic viral integration site 2A	231675	H93149	475	476	0.63	0.35	1.51
17	Sjogren syndrome antigen B	49970	H29484	477	478	0.53	0.32	7.86
18	Macrophage inflammatory protein 1b	205633	H62985	407	408	0.52	0.28	12.73
19	Monocyte chemotactic protein 1	768561	AA425102	395	396	0.46	0.11	213.89
20	Monocyte chemotactic protein 2	1911099	AI268937	479	480	undetectable	0.26	423.31
21	Endothelin 1	47359	H11003	397	398	undetectable	0.56	14.29

22	GRO2 /macrophage inflammatory protein 2a	153340	R50407	481	482	undetectable	0.66	12.16
23	Small nuclear ribonucleoprotein SM D 1	47542	H16454	483	484	undetectable	0.22	11.01
24	Hypothetical protein FLJ11296	491460	AA150443	55	56	undetectable	0.51	8.96
25	GRO1 / macrophage inflammatory protein 2 precursor	324437	W46900	485	486	undetectable	0.48	15.29
26	GRO1 / macrophage inflammatory protein 2 precursor	323238	W42723	485	486	undetectable	0.40	7.92
27	Lymphocyte adhesion molecule 1	149910	H00756	487	488	undetectable	0.47	4.92
28	Sex hormone-binding globulin	82871	T69346	409	410	undetectable	0.36	3.57
29	ESTs	898045	AA598952	205	206	undetectable	0.53	2.37
30	Hypothetical protein bA395L14	842794	AA486203	57	58	undetectable	0.45	n/d

Legend to Table 10

The last 3 columns show mRNA expression as a ratio between the conditions being compared. Of these three columns the first two show expression in hypoxia relative to normoxia, done separately in resting macrophages or activated macrophages. The final column shows expression in activated macrophages relative to resting macrophages (both in normoxia) as a ratio. n/d = not determined due to low signal intensities. IMAGE ID and accession descride the exact identity of the arrayed clones and do not describe full length cDNA sequence database entries.

TABLE 11: Other genes repressed by hypoxia in macrophages

						RATIO		
				SEQ ID		Hypoxia / Normoxia		Activated/ Resting
Row	TITLE	IMAGE ID	accession	PROTEIN	NUCL	(resting)	(activated)	(normoxia)
1	Annexin AI	208718	H63077	401	402	0.86	0.49	1.27
2	ATP-binding cassette, sub-family E (OABP), 1	1593311	AI002355	411	412	0.62	0.51	0.79
3	ESTs	855583	AA664228	165	166	0.61	0.52	0.73
4	Chaperonin / Tcp zeta 1	45233	H07880	413	414	0.59	0.55	1.27
5	Chaperonin TCP1, subunit 6A zeta1	45233	H07880	413	414	0.72	0.52	1.00
6	Colony stimulating factor 1 (macrophage)	73527	T55558	415	416	0.44	0.38	0.44
7	Colony stimulating factor 1 (macrophage)	1475574	AA878257	415	416	0.46	0.38	0.30
8	Dendritic cell protein (GA17)	563634	AA101348	417	418	0.59	0.53	0.97
9	G protein-coupled receptor 44	810403	AA464202	419	420	0.55	0.57	0.90
10	Heat shock 70kD protein 4	856567	AA633656	399	400	0.55	n/d	n/d
11	Keratin 6A	366481	AA026418	421	422	0.53	1.21	0.25
12	lymphocyte adaptor protein	294196	N71394	423	424	0.67	0.50	0.26
13	Neuro-oncological ventral antigen 1	2015354	AI362062	425	426	0.45	0.38	0.65
14	N-SMase / FAN	376644	AA046107	427	428	0.59	0.86	0.18
15	p67 myc protein	812965	AA464600	403	404	0.66	0.59	1.14
16	Peptidylprolyl isomerase F (cyclophilin F)	774726	AA442081	429	430	0.82	0.44	n/d
17	PLECKSTRIN	823779	AA490267	431	432	0.76	0.52	1.30
18	High affinity immunoglobulin epsilon receptor beta subunit	199185	R95749	433	434	0.65	0.59	1.22
19	High affinity immunoglobulin epsilon receptor beta subunit	79576	T62849	433	434	0.64	0.58	1.20

20	Ribosomal protein L44	884842	AA669359	435	436	0.73	0.50	1.36
21	Solute carrier family 6 No1	177967	H46254	437	438	0.62	0.54	1.38
22	Synaptopodin	178792	H49443	439	440	0.54	0.56	1.24
23	TERA protein	1521977	AA906997	441	442	0.44	0.34	0.46
24	TGF beta-stimulated protein TSC-22	868630	AA664389	443	444	0.56	0.72	0.83
25	Tubulin, beta, 2	1492104	AA888148	445	446	0.53	0.64	1.33
26	Calgranulin A	562729	AA086471	447	448	0.57	0.65	16.86
27	Replication factor C (145 KDa)	214537	H73714	449	450	0.66	0.50	2.80
28	Signal recognition particle 19 kD protein	754998	AA411407	451	452	0.63	0.49	2.77
29	Nucleoside phosphorylase	769890	AA430382	393	394	0.50	0.79	2.75
30	Transcription factor SUPT3H	1606865	AA996042	453	454	0.50	0.47	2.64
31	Proteasome component C9	399536	AA733040	455	456	0.64	0.58	2.09
32	Hypothetical nuclear factor SBBI22	868119	AA634213	35	36	0.75	0.50	0.73
33	DKFZP434I116 protein	207379	H58884	37	38	0.52	0.43	1.04
34	Hypothetical prot. FLJ10206	487921	AA045286	39	40	0.61	1.00	0.24
35	hypothetical protein FLJ10815	1506046	AA905628	41	42	0.92	0.55	0.88
36	Hypothetical protein FLJ11100	811590	AA454607	43	44	0.56	0.47	1.17
37	hypothetical protein FLJ2064	868161	AA633831	45	46	0.81	0.47	0.85
38	Hypothetical protein HSPC111	825695	AA504814	47	48	0.43	0.44	1.05
39	hypothetical protein LOC51251	379941	AA778116	49	50	0.56	0.55	1.06
40	Hypothetical protein LOC51251	770997	AA427715	49	50	0.56	0.74	1.47
41	cDNA FLJ13016 fis, clone NT2RP3000624	77483	T58743	59	60	0.58	0.54	1.55
42	DKFZp564D016 (clone DKFZp564D016	824665	AA482278	147	148	0.50	n/d	n/d
43	cDNA FLJ11302 fis, clone PLACE1009971	108351	T70612	149	150	0.75	0.49	1.36
44	NEDO FLJ10309 fis cl NT2RM2000287	810026	AA455267	151	152	0.57	1.20	0.28

45	Sequence from clone RP11-394O2 on ch 20	122147	T98503	153	154	0.51	0.99	n/d
46	ESTs (UniGene annotated)	731255	AA420992	155	156	0.67	0.53	0.86
47	ESTs (UniGene annotated)	434182	AA693797	157	158	0.46	0.37	0.77
48	ESTs (UniGene annotated)	788415	AA456437	159	160	0.50	0.52	0.89
49	ESTs (UniGene annotated)	49879	H28725	159	160	0.35	0.38	n/d
50	ESTs (UniGene annotated)	770954	AA429367	161	162	0.64	0.74	0.82
51	ESTs (UniGene annotated)	770935	AA434382	163	164	0.55	0.98	0.51
52	ESTs	34626	R44397	167	168	0.92	0.61	1.35
53	ESTs	1534589	AA923509	169	170	0.70	0.48	0.72
54	ESTs	417223	W87747	171	172	0.53	0.37	0.97
55	ESTs	854752	AA630167	215	216	0.64	0.54	0.87
56	ESTs	1569263	AA973568	173	174	0.52	0.45	0.62
57	ESTs	869440	AA679939	213	214	0.60	0.53	0.78
58	ESTs	123065	T98529	175	176	0.62	0.55	0.94
59	ESTs	364468	AA022679	177	178	0.59	0.54	1.30
60	ESTs	50635	H17921	179	180	0.45	0.43	0.94
61	ESTs	123858	R00766	181	182	0.50	0.50	0.75
62	ESTs	415195	W91958	183	184	0.54	0.59	1.28
63	ESTs	138865	R63694	185	186	0.56	0.66	1.32
64	ESTs	773308	AA425386	187	188	0.51	0.61	0.63
65	ESTs	1505857	AA909912	189	190	0.46	0.78	0.83
66	ESTs	122728	T99032	191	192	0.49	0.39	0.42
67	ESTs	202154	H52503	193	194	0.35	0.42	0.35
68	ESTs	502634	AA127017	195	196	0.52	0.88	0.54
69	ESTs	23005	R38647	197	198	0.51	n/d	n/d
70	ESTs	22500	T87233	199	200	0.55	n/d	n/d
71	ESTs	587398	AA130351	201	202	0.52	n/d	n/d

72	ESTs (ex-UniGene)	1610469	AA991868	207	208	0.54	0.53	0.41
73	ESTs (ex-UniGene)	81331	T60111	209	210	0.92	0.46	0.24
74	ESTs (ex-UniGene)	1425266	AA897090	211	212	0.55	0.75	0.19

Legend

The last 3 columns show mRNA expression as a ratio between the conditions being compared. Of these three columns the first two show expression in hypoxia relative to normoxia, done separately in resting macrophages or activated macrophages. The final column shows expression in activated macrophages relative to resting macrophages (both in normoxia) as a ratio. n/d = not determined due to low signal intensities. IMAGE ID and accession descride the exact identity of the arrayed clones and do not describe full length cDNA sequence database entries.

TABLE 12

		#1	#1	#1	#2	#2	#2	#3	#3	#3	#4	#4	#4	#5	#5	#6	#6	#6	#7	#7	#7	#8	#8	#8	#9	#9	#9	#10	#10	#10	#11	#11	#11
		NO	HY	HY	NO	HY	HY	NO	HY	HY	NO	HY	HY	NO	HY	NO	HY	HY	NO	HY	HY	NO	HY	HY	NO	HY	HY	NO	HY	HY	NO	HY	HY
			6hr	18hr		6hr	18hr		6hr	18hr		6hr	18hr		6hr		6hr	18hr		6hr	18hr		6hr	18hr		6hr	18hr		6hr	18hr		6hr	18hr
Seq ID	Clone																																
2	p1F12	1.26	0.83	1.12	2.59	2.41	2.84	1.36	1.42	1.75	0.41	0.35	0.41	0.70	1.59	0.48	0.46	0.71	0.41	0.45	0.26	0.42	0.70	0.65	1.55	1.34	1.68	0.80	1.06	0.95	1.21	1.68	1.38
4	p1F2	1.39	2.37	1.74	1.18	1.78	1.62	0.74	0.67	0.50	0.80	1.12	1.24	1.30	1.98	0.94	1.38	1.29	0.83	1.55	0.94	1.42	1.92	4.03	0.73	0.57	0.87	0.31	0.33	0.42	0.69	1.03	0.79
6	p1F10	1.41	1.07	0.83	1.57	1.71	1.71	1.60	1.92	1.67	0.79	0.66	0.56	0.69	1.48	1.17	0.94	1.50	0.98	2.39	0.54	0.59	0.77	0.87	1.01	0.88	0.72	0.77	0.91	0.76	1.86	1.90	1.62
8	p1F19	0.54	0.53	0.78	0.99	1.04	1.25	1.18	1.23	1.22	0.44	0.54	0.72	0.37	0.29	0.94	0.67	0.71	2.69	4.29	1.99	0.57	0.87	1.12	1.21	1.79	1.07	1.16	1.57	1.56	1.06	0.98	0.93
10	p1F8	0.63	1.34	1.17	0.87	1.04	1.04	0.53	1.07	1.09	0.50	1.20	1.96	0.32	0.99	0.74	1.21	1.69	0.60	2.33	0.81	1.22	2.39	3.16	0.91	0.91	1.82	0.33	1.03	1.98	1.33	2.23	3.62
12	p1F5	1.41	1.04	0.98	1.12	1.63	1.35	2.09	2.40	1.91	0.78	0.65	0.88	0.81	1.67	0.83	0.75	1.46	0.57	1.93	0.41	0.96	1.83	2.23	0.85	0.86	0.75	0.63	0.81	0.81	2.06	2.83	1.93
14	p1F18	1.34	0.69	1.02	1.43	1.65	1.90	1.98	1.62	2.17	0.74	0.55	0.72	0.40	0.57	0.74	0.70	0.90	0.31	0.40	0.27	0.78	1.21	1.00	1.33	1.41	1.00	1.16	1.67	1.54	1.04	1.01	1.04
16	p1F7	0.69	0.35	0.75	1.83	1.35	1.67	1.99	5.27	2.95	1.05	0.91	0.71	0.47	0.47	0.05	0.08	0.05	0.91	0.86	0.52	0.01	0.03	0.02	1.87	1.16	1.17	3.87	5.09	6.00	1.13	1.59	1.52
18	p1F21	0.68	0.58	0.50	0.85	0.85	0.74	0.80	0.66	0.52	0.61	0.66	0.63	0.27	0.59	2.16	5.39	6.25	3.00	5.81	5.25	2.78	6.02	8.14	1.90	2.11	0.96	0.87	1.43	1.29	0.92	1.34	1.01
20	p1F9	0.36	0.47	0.86	0.95	2.47	3.09	3.60	2.70	3.01	0.43	0.93	1.02	0.23	0.54	0.29	0.62	0.81	0.66	2.81	2.93	0.30	1.24	1.98	1.26	3.69	6.69	0.93	2.81	4.90	0.76	1.30	1.41
22	p1E13	1.76	1.30	0.95	1.04	1.78	1.01	0.59	0.66	0.55	0.89	0.65	0.72	1.04	1.50	3.73	3.36	3.44	0.77	0.91	0.52	4.94	5.66	7.44	0.80	0.76	0.83	0.48	0.48	0.53	1.25	1.37	1.45
24	p1D1	0.88	1.98	5.49	1.47	2.03	3.59	1.32	2.69	2.05	0.63	2.45	5.51	0.94	1.79	0.10	0.33	0.79	0.84	1.45	1.75	0.06	0.08	0.15	0.18	0.21	2.61	0.41	1.22	2.15	0.52	1.53	2.90
24	p1D2	1.03	0.99	3.89	1.76	3.50	4.79	0.73	1.87	1.78	1.07	2.22	4.16	0.66	1.01	0.13	0.23	0.36	0.45	0.69	1.35	0.14	0.14	0.20	0.91	1.82	6.00	0.71	2.33	2.12	1.15	2.37	4.15
26	p1D4	0.41	1.88	1.95	0.83	1.80	2.19	1.57	1.80	1.54	0.42	1.42	1.50	0.49	2.36	0.31	0.39	0.86	4.30	30.5	28.2	0.07	0.88	1.59	0.17	0.22	2.68	0.96	4.76	10.5	0.28	1.01	0.69
28	p1D9	1.16	3.13	0.93	1.52	0.80	0.85	4.04	4.86	2.60	1.23	1.45	1.28	0.65	1.14	1.37	0.94	0.93	2.44	6.15	2.16	0.70	0.60	0.74	0.54	0.39	0.50	0.91	1.08	1.35	0.74	0.78	0.77

30	p1D12	5.56	4.42	3.24	0.62	3.70	1.69	0.54	0.77	0.78	1.09	1.13	0.96	1.36	1.03	3.70	4.01	5.05	0.20	0.50	0.28	1.23	1.20	1.70	0.25	0.35	0.36	0.27	0.76	1.01	0.94	1.09	1.10
32	p1D15	0.81	0.54	1.48	1.10	1.39	1.05	0.74	0.86	0.69	0.77	0.90	0.81	0.38	0.73	0.54	0.65	1.10	1.18	1.98	1.14	0.33	0.44	0.53	1.73	1.72	1.69	2.13	4.23	8.70	1.24	1.31	1.89
34	p1D16	0.44	1.99	0.84	1.11	1.29	1.22	2.07	2.43	1.61	0.42	0.78	1.32	0.61	1.18	0.66	0.98	1.42	1.67	4.88	1.93	0.70	0.86	1.12	0.33	0.24	2.10	0.73	1.20	1.81	0.48	0.88	1.28
36	p1J13	1.15	0.62	0.54	1.24	0.91	0.84	1.94	1.73	1.66	1.08	0.75	0.50	0.66	0.69	0.51	0.39	0.46	1.61	2.09	0.97	0.58	0.55	0.60	2.18	1.53	0.88	1.41	1.42	1.10	1.49	1.21	0.96
38	p1I22	0.72	0.65	1.20	1.97	1.77	1.49	1.57	1.60	1.75	0.95	0.90	0.86	0.95	1.59	1.10	0.74	0.82	0.58	0.59	0.16	0.48	1.16	0.82	1.45	0.97	0.85	0.87	0.96	1.00	2.15	1.26	1.26
40	p1J6	0.91	0.96	1.04	1.08	0.99	0.96	0.97	1.21	0.87	0.99	1.04	1.07	1.18	1.60	1.10	1.29	1.48	0.60	0.33	0.39	1.79	1.70	1.50	0.88	1.23	0.75	0.63	0.48	0.75	0.98	0.93	0.99
42	p1I5	3.02	1.24	2.56	1.56	3.17	1.47	1.46	1.76	1.21	1.16	1.32	0.93	0.54	0.88	0.73	1.07	0.86	0.52	1.00	0.31	0.35	0.56	0.55	0.90	1.09	0.56	0.81	0.65	0.38	1.71	2.18	1.36
44	p1I13	8.65	6.65	3.11	0.71	3.20	0.89	0.96	1.11	0.84	1.92	1.66	1.08	1.25	0.71	2.09	2.43	3.69	0.66	0.22	0.09	0.80	0.86	0.71	1.71	0.85	0.82	1.23	0.92	0.65	0.77	0.81	0.53
46	p1I17	0.88	0.55	0.63	2.03	1.13	1.38	1.93	4.01	2.52	1.16	0.92	0.88	0.23	0.32	0.67	0.51	0.64	0.74	0.71	0.42	0.54	0.75	0.73	1.67	1.42	2.09	1.44	1.48	1.22	2.14	2.56	1.78
48	p1I15	2.06	1.31	0.49	1.19	0.53	0.43	1.88	1.66	0.97	1.47	0.74	0.91	1.24	1.86	0.61	0.34	0.31	2.06	1.98	0.44	0.29	0.24	0.22	2.30	1.31	0.96	1.57	0.94	0.41	0.94	0.80	0.45
50	p1I7	0.79	0.56	0.64	1.20	0.69	0.80	2.13	3.28	1.74	0.91	0.96	0.99	0.47	0.65	0.57	0.34	0.37	1.86	1.54	1.42	0.28	0.36	0.49	3.38	2.22	2.73	2.24	2.29	2.35	1.19	0.94	0.91
54	p1I4	1.26	0.51	0.35	1.21	0.95	0.47	1.04	1.45	0.93	0.90	0.43	0.29	0.73	0.82	1.40	1.18	1.05	1.83	1.14	0.40	0.74	0.78	0.93	2.06	1.20	0.52	1.57	0.90	0.39	1.25	1.21	1.06
56	p1I8	4.53	3.14	2.83	1.51	2.71	1.50	0.89	1.04	0.76	1.24	1.09	1.00	0.65	1.04	0.93	1.26	1.50	0.31	0.27	0.27	0.46	0.50	0.59	0.90	1.14	0.84	0.36	0.50	0.56	1.24	1.76	1.35
58	p1I16	0.70	0.51	0.41	2.31	1.46	1.60	3.37	2.91	2.73	0.51	0.51	0.43	0.76	0.64	1.03	0.82	0.99	10.6	17.9	4.96	0.51	0.73	0.42	0.83	0.79	0.42	2.34	3.52	2.38	1.54	1.61	1.06
60	p1I11	0.63	0.62	0.34	1.64	1.36	1.19	2.75	1.62	1.38	0.63	0.37	0.40	0.59	0.84	0.56	0.51	0.57	1.13	1.47	0.49	0.64	0.61	0.55	3.68	2.80	2.80	1.54	1.51	1.07	1.89	1.81	1.62

62	p1E8	0.67	0.47	0.71	1.41	1.14	1.22	1.59	3.29	1.99	1.08	1.07	0.95	0.25	0.40	0.52	0.48	0.66	1.23	1.31	0.97	0.41	0.63	0.57	1.93	1.25	1.44	1.50	2.35	2.34	0.98	1.19	0.98
64	p1E18	1.26	1.07	0.72	1.45	2.07	1.10	0.77	0.93	0.73	0.93	0.71	0.80	0.57	1.23	6.36	5.38	6.48	0.46	0.48	0.34	2.58	2.30	3.06	1.14	0.78	0.72	0.51	0.62	0.68	1.56	1.91	1.78
66	p1E16	0.84	0.56	1.06	0.96	0.95	0.88	1.90	6.76	5.00	2.43	2.67	2.25	0.32	0.49	0.62	0.81	1.01	3.17	3.38	1.75	0.16	0.53	0.90	0.18	0.22	0.17	0.75	1.05	1.15	1.25	1.08	1.31
68	p1D5	1.71	1.21	1.40	1.58	1.81	1.66	2.13	1.93	2.05	0.84	0.79	0.81	0.78	0.90	0.75	0.80	0.98	0.47	0.82	0.51	0.52	0.74	0.71	1.13	0.91	1.23	1.18	1.58	1.21	1.05	1.24	1.13
68	p1D6	0.24	1.42	2.51	0.28	0.61	0.93	1.77	1.57	1.85	0.25	1.35	2.82	0.34	0.52	0.61	1.49	2.56	1.76	11.9	9.56	0.51	1.35	2.15	0.10	0.13	1.08	0.39	1.28	3.59	0.26	0.39	1.00
70	p1E12	0.54	0.41	0.62	0.98	1.29	0.67	0.98	1.47	1.13	0.83	0.84	0.87	1.11	1.17	0.59	1.01	1.03	0.67	1.66	0.89	0.53	0.88	1.07	1.27	1.41	1.18	1.21	1.59	1.86	1.11	1.20	1.06
72	p1E10	1.09	0.63	1.03	4.31	3.17	5.08	2.27	4.17	3.76	1.03	1.01	1.06	0.75	0.74	0.74	0.79	0.91	1.62	1.31	0.79	0.60	0.78	0.79	0.74	0.70	0.48	2.12	0.88	1.20	1.37	1.53	1.27
74	p1C21	3.92	2.75	2.31	0.93	2.33	0.92	1.81	2.27	1.45	0.91	0.96	0.95	0.41	0.33	2.10	2.68	2.86	0.60	0.66	0.49	0.60	0.65	0.85	0.41	0.49	0.15	1.16	1.65	0.93	1.09	1.12	1.00
76	p1D10	0.45	0.88	1.49	0.60	1.80	1.63	1.34	2.61	2.82	0.34	1.48	1.83	0.65	1.21	0.36	0.49	0.89	1.45	2.68	2.94	0.35	0.35	0.53	0.75	1.64	3.48	0.80	4.59	6.54	0.50	1.17	1.22
78	p1D13	2.93	1.83	2.94	0.80	3.69	1.10	1.51	3.28	3.02	0.89	1.59	1.30	0.67	0.64	0.50	0.75	0.82	0.50	0.76	0.45	0.15	0.27	0.42	0.52	2.31	2.24	1.08	3.73	2.25	0.92	2.02	2.80
80	p1E9	1.54	0.89	1.48	1.18	1.80	1.53	0.68	0.82	0.60	0.60	0.64	0.73	0.92	1.62	4.20	3.97	6.19	0.25	0.42	0.18	2.33	3.63	5.01	0.93	0.80	0.85	0.57	0.61	0.61	1.58	1.37	1.17
82	p1F1	0.58	0.33	0.52	1.42	1.19	1.14	1.64	1.70	1.49	0.95	0.63	0.54	0.37	0.50	0.62	0.61	0.51	0.91	0.90	0.30	0.34	0.53	0.66	1.66	1.23	1.06	1.46	1.58	1.03	1.25	1.24	1.06
84	p1E7	1.20	1.29	1.63	0.12	0.18	0.22	1.40	1.03	0.71	2.36	2.17	2.85	4.89	6.75	0.08	1.07	0.86	0.87	2.06	1.32	0.03	0.75	1.20	0.04	0.05	0.04	3.09	2.60	3.60	0.60	0.70	0.74
86	p1E6	1.36	1.09	1.01	0.79	0.92	1.04	0.79	1.35	1.04	1.32	1.87	1.63	3.25	5.97	0.34	0.74	0.85	0.58	2.26	2.67	0.25	0.57	0.65	0.46	0.52	0.82	1.89	1.89	3.91	1.17	1.42	1.57
88	p2B1	1.51	0.97	0.80	2.39	2.31	2.25	1.06	1.22	1.18	1.10	0.77	0.51	0.72	1.19	0.60	0.50	0.77	0.97	1.30	0.60	0.65	0.87	0.56	44.4	43.0	40.8	1.00	0.82	0.90	2.60	2.73	2.05

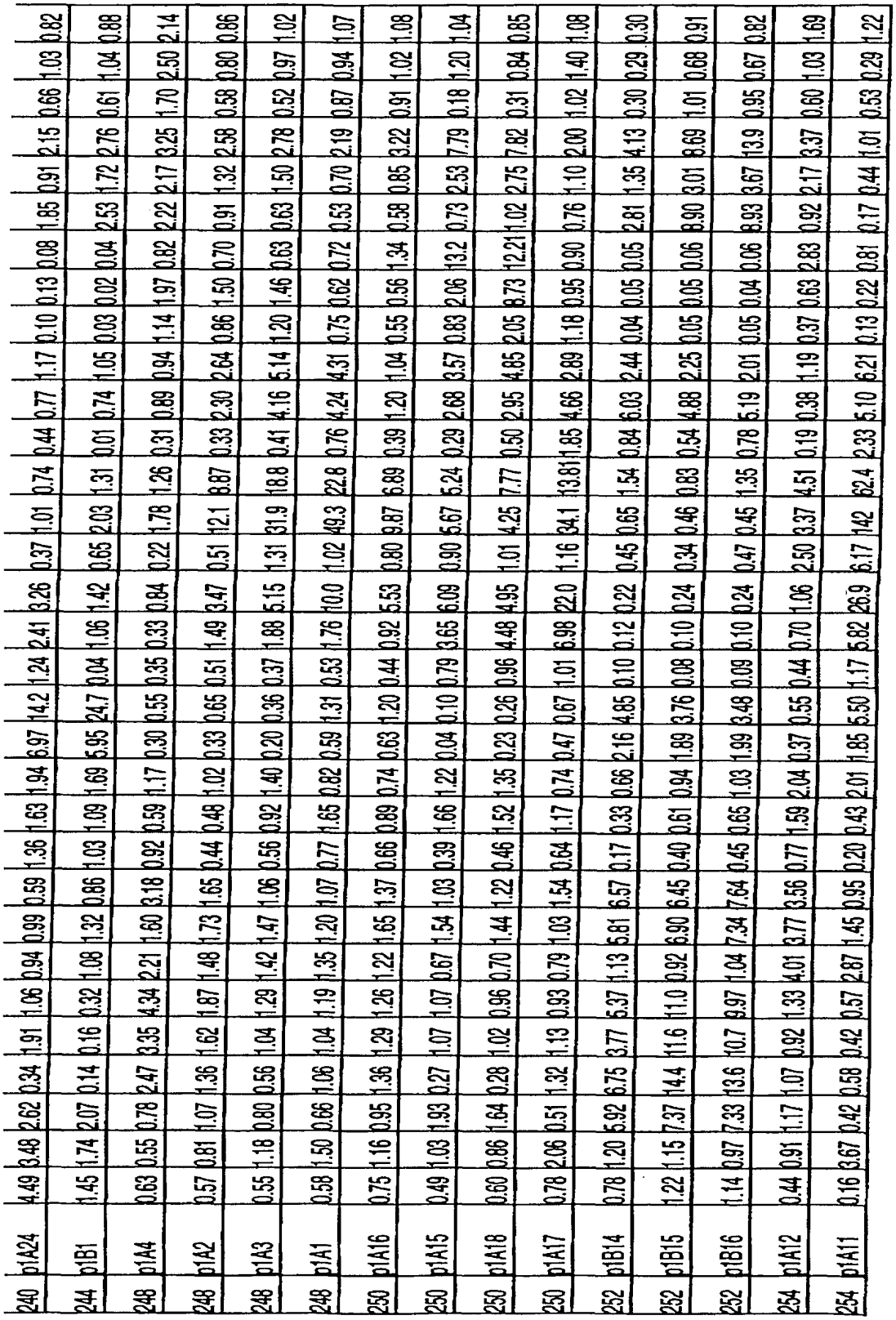
90	p1D14	0.67	0.82	1.64	0.60	3.42	1.80	1.78	5.24	3.57	1.07	2.33	2.11	0.68	0.67	0.29	0.74	0.52	0.58	1.23	1.29	0.24	0.75	0.72	3.09	9.75	4.95	2.47	5.03	3.55	0.79	2.49	2.20
92	p1D17	6.32	5.30	3.77	0.46	3.08	1.47	0.41	0.60	0.43	1.05	0.92	0.91	1.17	1.13	3.73	3.80	5.77	0.58	1.01	0.76	0.98	1.26	1.31	0.35	0.56	0.65	0.79	2.26	4.40	0.83	1.71	0.82
92	p1P14	0.43	0.66	0.74	1.22	1.60	1.14	1.00	1.53	1.32	0.49	0.73	1.15	0.43	0.69	0.21	0.45	0.68	2.63	11.8	5.90	0.14	0.71	0.84	1.46	1.93	3.86	4.65	15.4	34.0	0.82	2.07	1.12
94	p1C24	0.92	0.88	0.53	1.76	1.65	1.37	1.08	1.09	0.89	1.23	1.07	0.85	0.68	1.28	0.60	0.77	3.67	1.02	2.70	1.61	1.06	1.66	0.88	1.22	0.95	1.30	0.77	0.67	0.81	1.31	1.62	1.57
96	p1D3	0.62	1.56	1.13	1.83	0.85	0.73	3.09	4.01	3.43	0.29	0.52	0.71	0.15	0.28	5.72	5.67	5.80	5.79	9.49	5.80	3.32	3.26	5.79	0.36	0.29	0.54	0.38	0.42	0.77	0.67	0.93	0.98
98	p1E14	1.09	1.92	0.89	1.93	1.46	0.96	1.23	1.58	0.96	0.88	0.71	1.26	0.87	1.68	0.67	0.89	2.54	1.20	9.33	2.55	0.38	0.74	1.03	0.80	0.74	0.82	0.76	0.94	0.99	1.32	1.63	1.27
100	p1E20	0.63	0.61	1.12	1.47	1.34	0.99	1.45	1.94	1.49	0.68	0.88	0.98	0.19	0.34	0.36	0.43	0.37	1.33	1.44	0.65	0.23	0.43	0.33	0.97	1.30	1.16	0.94	1.89	1.78	1.12	1.51	1.34
102	p2A24	1.21	0.65	0.67	2.42	2.21	2.48	2.35	3.05	2.59	1.10	0.76	0.68	0.68	0.91	0.28	0.22	0.38	0.37	0.40	0.20	0.18	0.26	0.17	1.73	1.59	1.32	1.60	1.85	1.36	1.36	1.95	1.44
104	p1E17	0.94	0.56	0.99	0.95	1.55	0.96	1.93	2.41	2.37	0.89	0.88	0.80	0.53	0.73	0.84	1.15	0.90	0.29	0.39	0.24	1.96	2.03	2.06	1.27	1.47	0.91	1.13	1.31	1.33	0.97	1.19	1.01
106	p1E19	0.79	0.65	0.62	2.64	2.37	2.99	2.10	1.61	1.77	0.61	0.40	0.64	0.42	0.89	0.68	0.76	1.08	0.41	0.50	0.32	0.38	0.90	0.47	1.30	1.11	1.17	1.29	1.52	1.39	1.14	1.69	1.39
108	p1E15	2.82	1.46	2.04	1.42	2.65	1.00	0.92	1.10	0.78	1.83	2.00	1.73	0.82	0.72	1.21	1.35	1.27	1.12	1.01	0.85	0.52	0.65	1.11	0.44	0.56	0.30	0.28	0.29	0.32	0.93	1.05	0.75
110	p1E11	1.36	1.12	0.87	2.09	2.86	3.03	0.74	0.91	0.88	0.89	0.59	0.79	0.77	2.09	2.76	2.97	2.90	0.63	0.84	0.34	0.64	1.47	1.35	1.18	1.16	0.64	0.74	0.79	0.88	1.79	2.49	1.93
112	p1E23	1.19	0.65	1.55	1.24	2.11	1.42	0.75	1.15	1.02	0.86	1.09	1.09	0.35	0.40	0.31	0.36	0.48	0.88	1.02	0.73	0.30	0.55	0.66	3.17	2.93	2.98	1.21	2.36	2.53	2.23	2.61	2.23
114	p1E21	0.82	0.52	0.85	2.08	2.28	2.04	2.14	2.04	2.27	0.74	0.49	0.79	0.51	1.04	0.48	0.67	0.67	0.35	0.34	0.21	0.56	1.14	0.89	1.52	1.62	1.30	1.42	1.69	1.38	1.02	1.32	1.03
116	p1D23	0.95	0.47	0.68	1.21	1.62	1.30	0.99	1.17	1.10	1.39	1.20	1.05	0.35	0.49	0.28	0.27	0.34	0.80	0.85	0.66	0.39	0.49	0.53	2.49	2.28	1.52	1.02	1.77	1.19	1.25	1.21	1.17

118	p1D24	0.98	0.71	1.39	1.55	1.98	2.16	1.18	1.47	1.01	0.74	0.49	0.67	1.53	1.94	0.90	0.98	0.96	0.71	0.79	0.49	0.53	0.90	0.93	0.81	0.51	0.43	4.63	10.9	11.79	1.85	2.23	1.65
120	p1D22	0.70	0.89	1.98	0.89	2.66	2.13	0.71	1.40	1.45	0.72	1.61	2.10	0.26	0.65	0.22	0.96	0.84	0.92	2.62	2.82	0.32	1.26	2.00	2.03	7.51	5.60	0.91	3.88	6.11	0.62	2.09	2.39
122	p1E2	3.39	1.91	1.92	1.56	1.78	1.65	1.11	1.09	1.03	3.44	3.32	1.96	0.68	0.78	0.76	0.73	0.68	0.51	0.58	0.32	0.53	0.51	0.68	3.13	2.02	1.22	1.15	1.93	1.52	0.93	0.61	0.59
124	p1E1	2.91	1.31	2.14	3.07	6.21	2.25	1.08	2.11	1.28	1.02	1.01	1.21	0.56	0.80	0.64	1.16	1.25	0.29	0.42	0.29	0.28	0.47	0.91	1.03	1.24	0.88	0.32	0.61	0.60	3.03	4.39	2.33
126	p1E4	0.65	0.51	0.79	1.06	1.24	1.18	1.59	1.57	1.70	0.80	0.76	0.91	0.34	0.39	0.97	1.13	1.21	1.65	1.17	0.82	0.74	1.28	1.71	0.95	0.97	0.93	0.78	1.35	1.44	1.30	1.48	1.35
128	p1D18	8.78	5.01	4.93	1.12	5.44	2.18	0.58	0.80	0.50	1.25	1.25	0.99	1.15	1.18	1.34	1.93	2.33	0.61	1.12	0.59	0.21	0.47	0.44	0.47	0.56	0.34	0.81	0.66	0.93	1.67	2.06	1.43
130	p1D21	1.56	0.98	0.96	1.18	2.30	1.47	0.48	0.78	0.60	0.67	0.60	0.59	0.40	0.63	0.91	1.44	1.58	0.75	0.63	0.26	0.63	0.94	1.35	1.42	1.59	1.61	4.36	8.21	7.50	0.98	1.29	1.38
132	p1C22	3.70	2.08	2.77	0.94	2.43	1.34	0.92	1.26	0.74	1.85	1.25	1.49	0.91	0.77	2.36	2.86	3.39	0.24	0.24	0.21	1.34	1.44	2.22	0.54	0.74	0.36	0.34	0.38	0.45	0.93	1.16	0.89
134	p1C23	0.91	0.93	0.88	1.84	1.17	1.36	3.92	3.52	2.77	1.02	1.07	1.63	0.60	0.73	0.67	0.54	0.85	0.87	1.53	0.69	0.31	0.63	0.59	1.02	0.68	1.28	1.64	2.00	2.74	1.27	1.80	1.29
136	p1D11	0.54	1.09	1.35	0.73	1.57	1.74	2.78	4.49	3.68	0.60	1.22	1.63	0.80	1.83	0.22	0.74	0.90	0.33	2.31	1.23	0.26	1.00	1.34	0.38	0.41	1.04	0.49	2.07	2.79	0.86	1.77	1.60
138	p1E3	2.55	0.54	0.61	6.62	1.75	1.81	0.34	0.15	0.09	1.15	0.42	0.25	0.28	0.41	2.49	2.94	3.29	0.99	0.43	0.18	5.87	6.74	7.79	0.35	0.44	0.17	1.79	0.50	0.31	2.09	1.61	1.29
140	p1D20	0.75	0.93	1.16	1.35	1.72	1.18	1.63	2.62	2.78	0.57	0.54	0.75	0.43	0.69	0.66	0.77	0.78	1.34	1.76	0.65	0.34	0.71	0.72	2.43	2.48	1.46	0.66	1.80	1.75	3.27	3.69	4.17
142	p1E5	0.86	0.56	0.60	1.59	1.42	1.41	0.94	1.32	0.94	0.41	0.82	0.56	6.88	11.0	2.01	5.20	3.83	0.83	1.03	0.57	0.83	1.57	1.37	0.67	0.70	0.68	0.55	0.64	0.78	1.19	1.55	1.19
144	p1D19	3.52	1.95	2.39	1.10	3.29	1.54	0.96	1.23	0.82	1.87	1.69	1.80	0.59	0.66	1.50	2.04	1.97	0.16	0.12	0.14	0.80	1.04	1.08	0.46	0.58	0.25	0.49	0.46	0.59	1.34	1.62	1.22
146	p2A15	0.89	0.64	0.65	2.04	1.91	1.67	2.70	3.56	3.33	1.25	1.20	1.00	0.74	0.69	0.44	0.50	0.54	0.64	0.82	0.41	0.64	0.39	0.40	1.61	1.48	1.44	2.08	2.36	2.45	1.01	1.17	1.02

148	p1I14	0.96	0.54	0.67	1.30	0.68	1.11	2.36	2.45	1.69	1.10	0.90	0.47	0.20	0.24	0.38	0.26	0.26	2.20	3.06	1.28	0.15	0.27	0.19	1.67	1.28	0.67	1.87	2.43	1.77	1.06	1.00	0.68
150	p1I2	1.89	1.15	1.70	2.50	3.12	2.26	1.57	1.92	1.47	0.73	0.60	0.85	0.46	0.66	0.95	0.63	0.93	1.17	1.93	0.88	0.16	0.30	0.31	1.14	1.13	1.01	0.36	0.55	0.54	3.57	5.13	2.75
152	p1I12	2.00	1.52	1.68	0.91	1.02	1.40	0.66	0.88	0.62	1.81	1.36	1.48	1.96	2.49	2.13	2.37	2.71	0.54	0.27	0.32	2.90	3.25	2.42	0.62	0.85	0.44	0.36	0.34	0.40	0.87	1.00	0.87
154	p1I3	1.26	0.94	0.55	1.53	1.29	1.14	1.13	1.40	1.20	0.85	0.50	0.42	0.70	0.89	1.04	0.70	0.76	0.94	1.64	0.59	0.45	0.72	0.52	1.46	1.20	0.61	0.88	0.93	0.81	1.46	1.81	1.11
156	p1I10	1.82	1.30	0.97	1.15	1.65	1.18	0.59	0.72	0.53	1.89	1.64	1.05	0.75	1.07	0.76	0.71	0.85	1.50	1.17	0.73	0.92	0.90	1.14	4.02	4.49	4.46	0.33	0.26	0.34	1.22	1.28	0.89
158	p1H18	0.52	0.65	1.16	0.83	0.67	0.23	1.49	1.33	1.35	1.34	1.21	0.97	1.51	2.07	0.70	0.67	0.22	1.07	0.49	0.15	0.65	0.80	1.00	1.71	1.19	0.75	1.47	0.95	0.79	1.78	1.45	1.00
160	p1H24	7.02	5.55	3.73	0.83	3.66	1.22	1.04	1.32	0.77	1.27	1.71	1.53	0.89	0.92	3.02	4.58	4.74	0.93	0.84	0.50	0.76	0.89	1.07	1.06	0.87	0.78	0.90	0.62	0.50	1.24	1.24	0.87
162	p1E22	0.48	0.30	0.53	1.93	2.28	2.13	1.68	1.65	2.38	0.41	0.35	0.49	0.35	0.46	1.26	1.00	1.62	1.04	1.05	0.62	0.85	1.66	1.16	1.07	1.02	0.90	1.01	1.27	1.27	1.05	1.39	0.89
164	p1H21	0.82	0.56	0.66	3.63	2.47	3.49	0.83	0.96	0.82	0.64	0.67	0.76	5.64	10.2	6.34	16.3	12.1	0.34	0.94	0.49	0.32	0.82	2.11	0.63	1.14	1.03	0.80	1.43	2.23	1.28	1.97	1.15
166	p1I1	1.64	0.90	1.61	1.02	1.71	0.85	1.13	1.33	0.97	0.88	0.85	0.77	0.78	0.84	1.43	2.04	1.37	0.52	0.45	0.30	1.22	1.28	1.73	0.98	0.70	0.51	0.66	0.51	0.77	2.16	2.43	1.44
168	p1H14	0.86	0.45	0.71	1.62	1.10	0.78	1.65	1.91	1.77	0.87	0.87	1.00	0.32	0.46	0.39	0.37	0.35	1.14	1.18	1.02	0.30	0.42	0.37	3.00	3.19	1.59	1.71	2.48	2.09	1.35	1.56	0.99
170	p1H11	0.96	0.63	0.72	2.19	2.53	2.46	2.53	3.11	3.04	0.74	0.67	0.79	0.46	0.87	1.11	0.78	0.74	0.24	0.41	0.22	0.42	0.68	0.92	1.28	1.28	1.26	2.20	2.56	1.81	1.12	1.13	0.97
172	p1H17	0.58	0.69	1.17	0.65	0.64	0.17	1.67	1.53	1.54	1.36	1.39	1.11	1.30	1.73	0.63	0.71	0.24	1.28	0.58	0.15	0.77	0.80	0.85	1.58	0.98	0.67	1.43	1.10	0.82	1.49	1.02	0.71
174	p1H12	2.06	1.50	1.29	1.79	2.33	2.36	0.84	1.09	0.87	0.80	0.63	0.69	0.82	2.34	0.96	0.78	1.03	1.02	1.56	0.71	0.58	0.95	0.61	0.93	1.28	1.16	0.51	0.63	0.76	2.85	3.33	2.57
176	p1H7	0.70	0.64	1.12	1.21	1.38	0.75	1.66	1.47	1.38	1.10	1.04	0.79	1.27	1.86	0.52	0.55	0.31	0.35	0.20	0.11	0.54	0.71	0.76	1.81	1.40	1.00	1.11	0.77	0.96	2.27	1.45	1.10

178	p1H15	2.34	1.79	1.89	0.91	1.77	0.73	0.92	0.85	0.57	1.17	0.80	0.76	1.10	1.14	1.43	2.36	2.35	0.61	0.32	0.18	1.05	1.30	1.88	1.00	0.73	0.43	0.83	0.48	0.37	1.77	1.51	0.96
180	p1H20	0.42	0.53	0.98	0.91	0.81	0.24	1.47	1.48	1.33	1.26	1.07	0.88	1.04	1.83	0.73	0.76	0.25	1.42	0.56	0.18	0.72	0.91	0.93	1.69	1.41	1.01	1.29	0.91	0.81	2.25	1.41	1.08
182	p1H8	0.86	0.87	1.87	1.02	1.13	0.22	2.17	2.37	1.65	2.56	2.34	1.63	1.93	2.30	1.00	0.97	0.42	0.15	0.06	0.03	0.94	0.98	1.07	0.87	0.57	0.32	0.78	0.44	0.67	2.28	1.78	1.17
184	p1H16	0.68	0.42	0.54	1.59	1.56	1.68	1.28	1.01	0.97	0.25	0.14	0.29	1.47	2.48	0.62	0.81	0.76	0.85	0.99	0.58	0.93	1.24	0.88	1.55	1.06	1.70	0.63	0.51	1.04	1.51	1.13	1.21
186	p1H9	1.31	0.64	0.55	0.94	0.74	0.46	1.88	2.01	1.52	1.05	0.97	1.02	0.25	0.40	0.45	0.35	0.47	1.26	1.82	1.00	0.38	0.46	0.73	3.02	1.86	2.13	2.67	3.21	2.86	1.66	1.28	0.87
188	p1H23	1.10	0.60	0.60	1.28	0.97	0.67	1.96	2.81	2.33	1.20	0.93	0.86	0.40	0.52	0.39	0.34	0.38	1.16	1.05	0.99	0.38	0.46	0.53	4.59	3.73	1.67	1.90	4.02	2.27	1.30	1.08	0.77
190	p1H10	7.36	4.40	4.02	1.57	7.80	2.18	0.85	1.11	0.73	0.97	0.82	0.98	1.07	1.42	2.62	3.20	3.68	0.65	0.65	0.43	0.69	0.81	1.24	0.73	0.80	0.71	0.35	0.39	0.50	1.91	1.81	2.21
192	p1H6	8.14	6.12	3.46	0.48	4.02	1.26	0.41	0.47	0.29	1.51	1.62	1.06	1.84	1.16	4.77	5.40	6.08	0.16	0.13	0.10	1.87	1.88	2.03	0.17	0.25	0.13	0.13	0.10	0.12	0.85	1.17	0.85
194	p1H13	0.59	2.10	1.03	0.83	0.58	0.21	2.87	1.67	1.71	0.49	0.64	1.22	3.85	5.85	1.99	1.55	0.72	4.92	3.05	0.37	2.06	1.40	1.91	0.51	0.46	0.90	0.68	0.37	0.49	1.05	0.47	0.87
196	p1H19	0.47	0.46	0.54	1.12	0.54	0.16	2.12	2.58	1.50	1.53	1.40	1.04	1.65	1.82	1.03	0.80	0.80	2.90	2.66	0.49	0.59	0.89	1.56	0.62	0.44	0.82	2.07	1.40	0.59	0.86	1.61	0.95
198	p1G22	1.14	0.78	0.72	2.75	2.67	1.41	8.35	12.7	8.79	1.35	1.21	1.01	0.59	0.70	0.77	0.63	0.77	0.58	0.60	0.24	0.33	0.47	0.49	0.94	0.72	1.03	1.65	1.71	1.62	1.88	2.51	2.06
200	p1G21	0.99	1.01	0.55	2.02	1.48	1.10	1.54	1.13	0.80	0.78	0.65	0.60	0.72	1.35	1.28	0.69	0.74	1.03	1.34	0.49	0.38	0.57	0.73	0.92	0.65	1.04	1.47	1.29	1.15	1.20	1.47	1.03
202	p1H1	1.33	0.82	0.82	2.50	2.70	1.59	0.94	1.26	1.95	1.22	1.06	0.98	0.81	0.87	0.65	0.82	1.08	0.46	0.38	0.25	0.65	0.94	1.02	1.44	1.84	1.44	1.77	1.69	1.29	1.01	0.97	0.81
204	p1G20	0.74	0.48	0.50	1.54	0.66	0.36	1.64	1.29	1.03	0.99	0.84	0.84	1.34	1.19	1.29	0.98	1.09	1.85	1.50	0.64	1.27	1.46	1.03	0.85	0.67	0.37	1.51	1.05	0.70	1.01	1.11	0.85
206	p1H5	2.46	1.48	1.52	1.40	3.58	1.14	5.93	16.5	11.3	0.97	0.81	0.87	0.80	1.19	0.92	1.18	1.14	0.49	0.58	0.40	0.69	0.78	0.91	1.07	0.84	1.04	0.55	0.44	0.29	2.09	2.45	2.06

208	p1G19	0.44	0.52	0.79	0.88	0.81	0.30	1.75	1.77	1.18	1.41	1.37	1.06	1.76	2.43	0.75	0.64	0.29	2.03	1.39	0.36	0.48	0.51	0.35	1.14	0.90	1.76	1.78	1.49	2.25	1.00	1.01	0.56
210	p1H2	1.13	0.55	0.49	0.84	0.83	0.39	2.34	4.04	4.03	0.68	0.56	0.47	0.12	0.17	2.43	2.35	1.86	0.91	0.82	0.72	1.94	2.47	2.65	2.55	2.29	0.35	1.66	2.25	1.06	0.61	0.57	0.36
212	p1G18	0.41	0.82	0.75	0.95	0.63	0.22	2.50	2.28	1.45	0.59	1.02	1.02	1.67	2.11	1.29	0.91	0.90	3.11	3.14	0.50	1.10	1.18	1.51	0.54	0.81	1.17	1.05	0.84	1.08	0.79	1.11	0.74
214	p1H4	1.61	1.00	1.44	0.92	1.89	0.63	1.15	1.46	1.00	1.26	1.15	1.02	0.90	0.66	1.11	1.21	1.26	0.21	0.08	0.04	1.06	1.04	1.37	0.81	0.78	0.44	0.69	0.54	0.45	1.26	0.89	0.74
216	p1H3	1.28	0.68	1.11	1.13	1.55	0.48	0.87	0.90	0.56	1.01	0.77	0.66	1.05	0.88	2.01	3.04	2.12	0.84	0.35	0.14	1.34	1.57	2.53	1.32	0.67	0.44	1.11	0.48	0.44	2.21	1.52	0.90
222	p1P3	1.32	0.79	1.45	1.39	2.10	1.47	17.1	10.7	10.8	0.82	1.02	1.15	0.53	0.83	0.48	1.08	1.92	0.44	0.79	0.38	0.27	0.44	0.66	0.47	0.55	0.30	7.15	7.93	6.97	1.12	1.18	1.12
224	p1A9	1.09	2.80	2.72	1.01	1.15	2.43	3.64	5.80	3.79	0.32	0.88	1.62	0.59	1.33	0.24	0.44	0.79	1.08	2.70	3.21	0.06	0.35	0.47	0.16	0.20	1.42	0.79	1.64	3.82	0.72	1.78	3.33
224	p1A8	0.52	11.0	1.43	0.60	0.57	0.83	4.46	4.51	2.81	0.17	0.42	1.28	1.75	4.84	0.99	2.32	6.46	4.52	37.2	24.2	0.63	1.17	1.40	0.13	0.14	0.44	0.27	0.49	1.37	0.39	0.56	1.59
226	p1B17	1.04	24.7	2.25	1.16	1.22	1.25	1.08	0.99	0.81	0.69	0.81	0.79	1.50	2.35	2.53	2.56	6.59	27.2	223	109	0.51	1.00	0.68	0.81	0.85	0.86	0.83	1.22	2.43	0.86	1.55	1.09
228	p1O20	0.58	1.42	5.10	0.73	4.10	7.70	0.63	0.84	1.02	0.74	2.32	2.75	0.35	0.96	0.06	0.10	0.27	4.25	7.39	5.74	0.09	0.44	0.44	1.19	14.2	31.1	5.43	8.1	15.1	0.68	2.46	5.18
230	p1B2	0.66	1.96	5.58	0.58	1.02	1.50	1.53	0.95	0.87	0.57	2.55	4.40	0.56	1.20	0.25	1.03	0.99	7.01	22.2	28.8	0.33	0.98	1.70	0.44	0.54	2.32	0.67	4.52	13.0	0.51	1.10	1.44
232	p1B3	1.24	1.35	3.82	1.78	2.17	4.64	3.30	3.42	2.96	0.79	1.01	1.32	0.10	0.26	0.15	0.40	0.85	0.33	1.27	1.04	0.09	0.42	1.08	0.33	0.41	3.73	0.97	1.72	1.54	0.84	1.63	2.88
236	p1B19	1.34	1.54	6.26	1.28	0.89	2.48	9.63	8.26	6.08	0.66	2.09	5.36	0.72	1.85	0.01	0.03	0.07	0.36	2.23	1.77	0.01	0.03	0.06	0.01	0.01	0.01	1.23	0.69	3.23	0.80	2.03	1.54
236	p1B18	0.65	18.3	2.73	1.68	1.06	2.54	26.2	10.0	4.27	0.19	0.57	5.12	2.30	16.1	0.16	0.34	0.89	2.35	29.2	13.7	0.20	0.34	0.39	0.17	0.17	0.15	0.21	0.17	0.69	1.36	0.66	1.75
238	p1N17	2.81	1.63	2.01	1.24	3.19	1.83	4.80	0.87	0.57	1.48	1.29	1.16	0.47	0.59	1.06	1.27	1.67	3.14	3.81	8.23	0.41	0.43	0.63	0.35	0.37	0.23	0.16	0.18	0.21	0.71	0.86	0.73



256	p1A13	0.34	2.14	1.10	0.42	0.36	0.65	3.57	3.81	3.15	0.19	0.79	1.98	0.34	0.60	1.24	1.86	3.13	1.49	4.12	4.95	0.92	1.18	2.38	0.06	0.06	1.27	0.29	0.81	2.00	0.21	0.36	1.05
258	p1A14	0.27	5.92	0.30	0.40	0.45	0.71	7.23	2.92	2.30	0.08	0.18	1.06	2.39	6.30	4.94	7.07	7.75	6.29	28.0	29.3	4.13	5.41	7.32	0.17	0.24	0.33	0.11	0.23	0.77	0.59	0.39	0.89
260	p1A19	0.56	2.88	0.45	0.99	0.90	1.33	2.11	1.14	0.73	0.45	0.53	0.82	1.46	4.56	4.90	1.37	15.7	1.21	33.7	4.98	0.74	5.11	2.30	0.68	0.81	0.61	0.43	0.56	0.52	0.86	0.64	0.89
262	p1A20	0.20	8.18	0.34	0.36	0.40	0.68	14.1	8.24	6.34	0.06	0.27	1.66	1.38	3.74	2.34	3.16	4.93	3.33	10.0	7.74	0.98	1.70	2.70	0.08	0.08	0.12	0.08	0.14	0.57	0.38	0.18	1.26
264	p1A22	0.78	1.44	2.34	0.52	1.40	1.52	2.59	3.81	2.38	0.46	1.47	1.92	0.77	0.98	0.19	0.56	0.87	0.84	1.41	1.18	0.07	0.24	0.32	0.61	1.92	3.26	2.03	4.60	4.51	0.75	2.15	3.17
266	p1A23	0.71	2.10	1.41	0.07	0.06	0.13	3.12	2.80	1.63	1.06	1.94	2.95	7.39	18.1	0.03	0.95	1.14	1.34	4.67	2.78	0.02	0.79	1.41	0.01	0.01	0.01	1.17	0.96	2.10	0.13	0.23	0.21
268	p1B21	3.08	1.82	2.69	0.59	3.22	0.71	0.56	0.66	0.41	1.48	1.45	0.75	0.77	0.99	96.6	117	165	0.17	0.31	0.17	1.35	3.24	55.7	0.37	0.40	0.27	28.9	30.2	86.8	0.90	1.00	0.67
268	p1B20	0.72	0.85	0.77	1.17	1.27	1.05	0.74	1.03	0.57	0.80	0.77	0.64	0.61	1.25	1505	1742	2952	1.05	3.07	1.20	17.5	32.8	682	0.76	0.85	1.29	45.1	42.8	224	1.14	0.96	1.15
270	p1C17	0.38	0.91	0.47	1.39	0.63	1.21	3.27	2.63	2.74	0.37	0.28	0.59	0.21	0.18	3.94	4.47	5.82	3.02	5.66	2.13	4.54	4.06	4.88	0.46	0.55	0.59	0.80	0.95	1.70	0.92	0.83	1.03
270	p1C18	0.42	0.76	0.51	1.51	0.99	1.69	2.88	2.45	2.64	0.32	0.31	0.54	0.16	0.27	4.76	5.23	8.06	2.62	7.14	2.17	3.71	3.18	4.70	0.45	0.66	0.69	0.67	1.03	1.59	0.91	1.00	0.96
272	p1D8	2.74	2.48	2.75	0.99	2.72	1.61	0.74	0.78	0.74	1.31	1.08	1.01	0.84	0.91	2.08	2.03	2.31	0.09	0.17	0.18	1.11	1.09	1.50	0.62	0.97	0.73	0.27	0.44	0.80	1.16	1.31	1.07
274	p1A10	0.13	1.74	1.78	0.33	2.02	1.93	3.00	3.12	2.80	0.20	1.69	2.86	0.06	0.27	0.30	1.81	3.43	0.14	2.68	4.91	0.12	0.78	1.90	0.53	0.36	6.58	0.90	5.24	22.3	0.10	0.91	2.13
276	p1G24	1.41	1.31	1.91	3.09	4.24	2.63	0.92	1.60	0.93	1.14	1.39	1.75	0.14	0.30	0.40	0.70	1.02	0.42	1.73	0.82	0.17	0.45	0.48	0.62	1.04	1.26	0.48	1.02	1.36	2.79	4.68	4.49
278	p1G23	2.81	1.41	1.97	1.06	2.80	1.23	0.90	1.14	0.62	0.94	0.85	0.80	0.37	0.51	2.52	1.37	2.13	0.90	2.33	0.80	0.73	1.13	1.75	0.66	0.90	0.81	0.44	0.84	0.95	1.72	2.05	1.83
280	p1G5	0.34	0.86	1.46	0.56	1.70	1.61	0.63	1.16	1.35	0.43	1.23	1.77	0.21	0.53	0.21	0.97	0.90	1.21	3.69	3.47	0.45	1.28	2.11	0.89	2.10	4.01	0.68	2.58	5.12	0.36	1.31	1.57

282	p1G7	0.74	0.61	0.80	1.25	1.26	1.03	1.13	1.77	1.39	0.87	1.28	1.54	0.37	0.56	0.36	0.61	0.52	1.12	1.85	1.19	0.23	0.40	0.50	1.27	0.92	1.35	1.62	2.39	1.90	0.94	1.54	1.32
284	p2A23	1.79	1.24	1.15	1.81	2.26	1.79	1.11	1.28	0.95	1.26	0.87	0.77	0.77	1.43	0.76	0.91	2.36	0.59	0.61	0.40	0.32	0.40	0.35	1.32	1.14	0.79	0.85	0.76	0.66	1.68	2.18	1.53
286	p1G1	0.54	0.39	0.81	1.68	2.29	3.15	1.05	1.41	1.33	1.85	2.65	1.71	0.35	0.58	0.21	0.33	0.35	0.53	0.85	0.75	1.04	1.89	1.39	1.17	1.16	0.98	0.74	0.91	0.95	1.16	1.20	1.52
288	p1G15	7.04	5.26	3.09	0.70	3.29	2.10	0.57	0.77	0.55	2.10	1.82	1.03	1.54	1.44	3.27	3.54	4.87	0.34	0.56	0.36	1.92	1.93	1.93	0.33	0.63	0.51	0.20	0.28	0.32	0.80	1.05	0.89
290	p1F23	0.90	0.42	1.09	1.89	1.13	1.09	1.22	2.57	1.79	1.10	0.88	0.70	0.42	0.55	0.33	0.40	0.32	0.84	0.77	0.70	0.29	0.32	0.32	1.50	1.79	1.29	2.59	2.59	1.90	1.67	1.38	1.16
292	p1G8	1.02	0.70	1.33	0.86	3.03	1.31	1.15	3.36	2.20	1.04	1.93	1.56	0.33	0.71	0.23	0.35	0.43	0.49	0.79	0.86	0.20	0.62	0.52	13.4	14.8	10.4	1.32	4.10	3.54	0.96	2.78	2.43
294	p1G13	8.69	6.51	4.23	1.06	4.37	2.12	0.37	0.47	0.36	1.39	1.41	0.82	1.03	1.17	5.78	7.17	9.42	0.46	0.47	0.43	2.39	2.87	3.25	0.15	0.16	0.13	0.28	0.43	0.64	0.79	1.19	0.96
296	p1G10	1.01	0.61	1.52	1.69	1.76	1.65	2.01	3.58	2.49	1.32	1.13	0.99	0.44	0.65	0.44	0.62	0.49	0.67	0.68	0.40	0.30	0.44	0.37	1.46	0.96	0.72	2.48	2.48	1.88	1.56	1.51	1.21
298	p1F24	1.17	0.70	1.01	9.63	5.52	8.15	0.56	0.87	0.56	12.8	11.5	14.2	0.12	0.15	0.11	0.14	0.08	2.07	1.62	1.89	0.13	0.17	0.19	0.45	0.58	0.36	1.18	1.06	1.55	5.29	6.20	4.26
300	p1G2	1.69	1.00	1.36	5.01	4.65	3.35	1.04	1.47	0.83	1.28	1.39	1.24	0.36	1.37	0.42	0.48	0.62	0.61	0.84	0.43	0.28	0.29	0.48	0.89	0.65	1.21	0.91	0.92	0.69	2.10	2.84	2.62
302	p1G11	0.53	0.42	0.42	0.55	0.86	0.65	1.43	3.83	3.56	0.45	0.36	0.43	1.21	1.55	0.29	0.53	0.65	3.60	4.27	4.45	0.16	0.26	0.20	6.96	8.65	6.54	11.3	18.7	26.3	0.98	1.30	1.18
304	p1G16	0.53	0.29	0.55	1.58	1.64	1.65	1.10	2.51	1.64	0.87	0.64	0.71	0.41	0.74	0.23	0.34	0.36	0.71	1.07	0.87	0.87	1.07	1.38	8.25	8.61	5.74	0.97	2.34	3.68	1.07	1.21	1.14
306	p1G9	1.32	0.61	0.77	1.07	1.78	0.79	0.74	1.22	0.96	0.95	0.85	0.66	0.79	1.09	0.94	1.06	1.43	1.28	1.06	1.00	0.49	1.14	1.76	0.85	0.66	0.52	1.39	1.80	2.13	0.98	1.04	0.96
308	p1G4	0.83	0.42	0.92	1.67	3.65	9.50	0.69	0.62	0.54	1.48	0.87	1.17	0.49	0.74	0.71	1.39	2.65	0.95	1.70	1.60	0.56	0.77	0.78	2.02	0.90	0.45	1.44	3.73	5.27	4.99	7.58	9.71
310	p1G14	8.40	5.55	4.36	0.70	4.34	1.75	0.47	0.56	0.36	1.14	0.98	0.90	1.30	1.11	3.49	4.03	4.68	0.15	0.16	0.13	1.08	1.48	1.63	0.33	0.49	0.25	0.17	0.18	0.18	0.94	1.57	1.09

312	p1A6	0.30	0.59	2.57	0.31	1.10	1.88	1.02	1.31	1.44	0.56	1.50	2.81	0.16	0.24	0.23	0.80	1.29	0.55	2.17	2.92	0.15	0.91	1.99	0.99	2.21	1.94	1.18	3.56	5.14	0.40	1.08	1.87
312	p1A5	5.61	2.87	4.93	1.15	4.00	2.95	0.60	0.87	0.47	1.77	1.64	1.79	0.56	0.55	1.70	3.28	3.93	0.19	0.23	0.22	0.98	1.78	3.76	0.25	0.33	0.24	0.15	0.20	0.26	1.25	1.65	1.39
314	p1B9	1.14	19.9	3.20	0.86	2.05	1.01	1.01	1.35	1.08	0.63	3.53	6.52	0.75	2.19	1.86	2.87	4.45	0.17	0.47	0.17	5.52	18.9	19.0	0.16	0.16	0.41	0.29	0.57	4.92	0.32	0.61	1.23
314	p1B6	0.69	18.7	1.14	0.59	0.72	0.92	0.70	0.54	0.61	0.59	0.89	1.01	1.54	4.45	2.13	3.09	8.12	1.22	2.38	1.81	10.0	25.1	16.0	0.60	0.57	0.58	0.99	1.38	2.38	0.79	0.54	0.87
314	p1B8	1.11	35.1	0.81	0.74	1.23	1.02	1.15	1.35	0.91	0.57	1.27	3.41	0.85	3.57	2.53	3.71	7.34	0.64	1.60	1.29	5.86	21.3	21.4	0.55	0.47	0.46	0.39	0.44	1.19	0.43	0.55	1.38
314	p1B7	0.60	34.9	1.17	0.59	0.74	0.80	0.90	0.80	0.72	0.48	0.84	1.76	1.94	4.62	4.60	7.31	12.00	1.15	2.78	1.89	21.1	41.1	42.4	0.46	0.44	0.57	0.75	0.85	1.97	0.46	0.40	0.97
316	p1G17	0.85	1.53	0.92	1.10	0.83	1.05	2.04	1.86	1.78	1.40	2.19	2.22	0.81	0.87	0.46	0.74	0.93	1.06	1.46	0.76	0.24	0.44	0.99	1.10	0.77	0.82	2.00	2.84	3.17	0.96	0.97	1.00
318	p1G3	0.38	0.39	1.02	0.76	1.16	0.87	1.38	1.89	1.71	0.81	0.73	1.00	0.81	2.03	0.24	0.48	0.30	1.65	1.68	1.50	0.58	0.86	1.02	0.95	0.99	1.52	1.23	1.74	3.24	1.28	0.97	1.78
320	p1F22	0.94	0.56	0.75	1.15	1.24	1.60	2.56	3.11	2.46	1.10	1.70	1.67	0.45	0.47	0.29	0.31	0.35	0.74	0.85	0.83	0.16	0.27	0.31	1.51	1.59	0.85	2.54	3.06	3.72	1.03	1.01	0.75
322	p1G12	0.29	0.41	0.96	0.99	1.95	1.56	0.80	1.75	1.22	0.54	0.87	1.24	0.12	0.31	0.16	0.30	0.39	1.53	2.64	2.85	0.33	0.51	0.84	1.69	3.85	4.80	0.83	3.71	5.25	1.74	2.60	3.23
324	p1F11	1.49	0.94	1.01	1.16	1.64	1.56	0.64	0.91	0.71	0.84	0.69	1.00	0.77	1.16	0.53	1.01	0.89	0.64	0.85	0.57	0.98	1.77	1.64	2.48	2.83	1.47	0.46	0.74	0.73	1.62	1.80	1.81
326	p1F16	2.63	0.62	0.65	6.26	1.77	1.43	0.39	0.16	0.13	1.07	0.39	0.30	0.29	0.30	2.73	3.22	3.38	1.02	0.52	0.21	7.19	6.39	9.11	0.31	0.33	0.16	1.44	0.39	0.29	1.68	1.44	1.20
328	p1F14	0.75	0.39	0.89	0.97	0.92	0.84	1.29	1.35	1.36	0.93	0.90	1.03	0.54	0.98	0.16	0.22	0.30	1.52	3.98	3.61	1.00	1.72	1.66	0.06	0.07	0.07	3.27	4.66	10.6	1.25	1.05	1.46
330	p1F17	1.52	0.95	1.82	5.53	3.68	5.49	0.24	0.32	0.33	2.14	2.44	2.18	0.86	1.66	0.22	0.92	1.32	11.7	16.0	9.79	0.04	0.27	1.01	0.06	0.07	0.07	0.12	0.12	0.23	1.19	2.33	1.66
332	p1C2	0.26	0.31	0.31	1.00	1.28	1.53	5.09	13.6	16.1	0.17	0.21	0.31	0.19	0.37	0.67	1.53	2.00	0.60	2.84	1.53	7.05	9.91	9.48	0.95	18.0	39.5	0.27	1.57	3.32	0.52	0.80	0.64

334	p1F3	1.64	1.06	1.20	1.52	1.52	1.41	1.05	1.55	1.28	1.26	1.02	0.99	0.71	1.05	0.46	0.50	0.87	0.56	0.75	0.45	0.40	0.64	0.66	1.14	0.99	0.74	0.79	1.38	1.14	1.82	2.15	1.65
336	p1F20	0.69	1.35	1.32	1.10	1.16	1.03	1.73	2.66	2.42	0.34	0.64	0.76	0.86	1.82	0.53	0.87	0.98	2.60	4.08	2.62	1.11	1.52	2.07	0.27	0.27	0.71	0.37	0.71	1.14	0.70	0.89	1.08
338	p1F6	1.92	1.67	2.74	0.11	0.17	0.28	1.73	1.30	1.02	1.80	2.33	2.74	5.33	7.33	0.04	0.73	0.91	0.45	1.45	0.91	0.01	0.45	0.61	0.04	0.05	0.04	2.18	2.32	3.68	0.92	1.03	0.84
340	p1F4	1.50	1.06	0.87	1.14	1.59	1.15	0.68	1.05	0.83	0.92	0.72	0.66	0.94	1.85	2.78	6.20	5.14	2.83	2.10	1.95	0.44	0.65	0.99	0.87	0.89	0.78	0.73	0.62	0.82	1.34	1.50	1.15
342	p1F15	1.29	0.82	1.10	1.97	1.87	1.33	1.71	1.75	1.30	0.62	0.78	0.74	0.34	0.81	0.63	0.83	1.06	0.79	1.22	0.71	0.35	0.56	0.84	1.57	1.93	0.90	1.16	1.46	2.33	1.36	1.38	0.93
344	p1F13	0.32	1.18	2.65	0.54	3.15	2.25	0.74	1.19	1.19	0.80	3.41	4.43	0.21	0.49	0.17	0.52	0.71	0.43	1.58	1.80	0.28	1.94	2.63	2.51	14.4	12.9	0.82	6.59	7.42	1.07	3.89	3.99
346	p1A7	0.74	0.60	0.50	0.87	0.63	0.80	0.85	0.95	0.69	0.71	0.57	0.76	0.73	1.22	1.59	1.11	2.67	5.16	9.61	4.32	1.15	2.12	2.18	0.99	0.37	0.61	1.58	1.30	1.60	1.86	2.36	1.59
348	p1A21	1.35	0.89	1.29	1.27	1.17	0.98	0.85	1.49	1.32	1.07	1.31	1.17	0.52	0.49	0.18	0.22	0.24	0.86	1.13	1.08	0.18	0.26	0.36	0.61	0.33	0.38	1.19	1.97	1.36	2.27	2.95	3.16
350	p1B5	1.08	0.94	3.22	1.73	1.39	3.45	2.08	2.24	1.78	0.34	0.55	1.57	0.10	0.17	0.07	0.13	0.10	0.90	1.37	1.04	0.02	0.03	0.06	0.18	0.30	1.85	1.47	1.82	3.02	1.66	3.19	3.40
350	p1B4	0.71	7.10	2.98	2.43	1.47	3.12	10.3	7.97	5.70	0.48	0.93	2.72	0.65	1.12	0.44	0.75	0.87	3.69	9.15	4.61	0.25	0.22	0.48	0.32	0.30	0.80	0.52	0.79	2.38	1.93	1.75	5.01
352	p1B12	1.74	1.15	1.23	0.70	1.53	0.93	0.60	0.57	0.30	0.94	0.88	0.89	0.34	0.37	1.15	1.50	1.82	0.74	0.84	1.09	0.75	1.02	0.97	0.49	0.64	0.37	1.28	1.90	1.56	1.10	1.37	1.41
352	p1B11	0.21	0.17	0.40	1.25	1.64	1.18	1.14	0.66	0.45	0.94	1.02	1.31	0.28	0.30	0.27	0.64	1.45	1.42	2.25	1.85	0.06	0.10	0.22	0.93	1.11	1.39	2.23	3.90	3.56	2.04	2.69	3.58
352	p1B10	0.46	0.97	0.71	1.31	0.94	1.06	1.92	2.27	1.82	0.51	0.43	0.63	0.86	2.71	0.44	0.99	3.03	1.34	6.67	4.03	1.05	1.41	1.44	0.72	0.82	0.98	0.55	0.67	0.88	0.90	1.41	1.05
354	p1B13	0.93	0.99	1.06	0.85	1.01	0.91	0.77	0.97	0.68	1.00	1.03	1.02	1.22	1.61	1.20	1.42	1.66	1.24	1.26	0.83	1.96	1.86	2.20	0.55	0.76	0.41	0.31	0.29	0.39	0.98	0.94	1.04

356	p1B22	3.21	1.60	3.89	1.04	0.81	0.97	3.14	4.98	4.06	1.67	1.55	1.15	0.20	0.21	0.30	0.34	0.29	2.78	2.80	2.50	0.05	0.06	0.07	0.13	0.11	0.04	0.28	0.87	0.39	3.93	3.62	4.04
358	p1B23	0.36	0.52	1.08	0.80	1.09	1.17	0.93	0.66	0.85	0.27	0.52	0.82	1.05	1.83	17.2	12.5	8.51	1.84	6.94	6.08	1.79	2.46	3.57	0.49	0.59	0.97	0.52	1.43	4.14	0.58	0.86	0.83
360	p1B24	0.54	0.24	0.51	0.67	0.52	1.09	1.88	2.32	1.98	0.65	0.54	0.70	0.34	0.34	0.86	1.59	1.27	1.32	1.41	1.33	0.92	1.14	1.14	4.95	2.46	1.92	2.93	3.67	5.83	0.80	0.98	0.76
362	p1C3	0.71	0.68	0.77	2.10	0.92	1.22	4.25	2.15	1.97	1.07	1.18	1.09	0.46	0.54	0.48	0.57	0.55	1.86	2.73	1.77	0.73	1.09	1.29	0.18	0.10	0.36	1.15	1.00	1.09	1.01	2.33	1.40
364	p1C4	0.80	0.81	0.75	1.82	1.94	1.74	1.18	1.08	1.02	0.88	0.77	0.78	0.73	1.04	0.79	1.02	1.78	0.92	6.16	1.47	0.55	0.81	0.71	1.06	0.88	0.96	0.67	0.66	0.78	1.44	2.81	1.64
366	p1C5	0.52	0.67	1.21	0.93	0.89	1.07	1.80	2.92	2.03	0.70	1.42	1.68	0.58	1.01	0.61	0.85	0.89	0.72	1.39	1.15	0.39	0.96	1.04	1.46	1.37	1.15	1.58	3.45	4.04	0.73	1.21	0.77
368	p1C6	0.41	3.01	0.46	0.86	0.85	0.76	1.67	1.69	1.30	0.51	0.72	0.81	0.97	2.40	1.15	4.75	19.92	1.79	28.4	14.2	2.36	5.29	5.01	0.57	0.94	1.90	0.38	0.60	1.00	0.57	0.66	0.96
370	p1C7	0.93	2.60	0.63	0.74	0.89	0.75	3.35	1.93	1.49	0.61	0.93	0.83	1.81	2.86	1.92	1.25	1.69	2.98	11.76	3.31	1.90	1.61	1.45	0.68	0.64	0.58	0.51	0.85	0.76	0.84	1.00	0.74
372	p1C8	0.11	0.51	0.83	1.17	1.79	2.54	1.93	2.11	1.46	0.07	0.82	0.84	0.61	1.54	0.89	0.77	1.04	1.70	7.73	8.58	0.20	0.66	0.98	0.04	0.54	3.98	3.29	9.13	21.3	0.23	1.77	1.66
374	p1C9	1.04	1.00	1.67	1.31	1.62	1.61	1.18	2.32	1.60	0.75	0.86	0.89	0.42	0.57	0.57	0.60	1.00	0.81	1.62	1.21	0.84	0.80	1.01	0.46	0.38	0.49	0.92	1.27	1.68	1.15	1.33	1.56
376	p1C10	0.65	0.58	0.43	1.29	1.10	1.42	0.93	1.48	1.17	1.07	0.93	0.84	0.68	0.84	14.6	15.2	28.7	0.64	1.66	0.81	1.18	2.19	2.92	0.89	1.18	1.55	0.76	0.77	1.09	0.89	0.94	0.70
378	p1C11	0.69	1.30	0.86	1.59	0.56	0.90	2.64	2.63	2.42	0.45	0.52	1.32	0.79	1.37	0.90	0.59	1.45	1.73	11.0	5.75	0.58	0.87	0.78	0.13	0.07	0.56	0.81	1.46	3.60	1.00	1.63	1.11
380	p1C12	1.00	1.39	0.78	1.31	1.07	1.31	2.18	1.86	1.29	0.75	0.91	1.05	0.97	2.57	0.55	0.88	3.79	0.76	24.4	7.12	0.84	1.64	0.79	0.86	0.91	0.84	0.76	0.97	1.41	1.05	0.81	1.04
382	p1C13	0.61	6.60	1.61	0.78	0.80	0.90	3.98	3.29	2.33	0.73	2.76	6.00	0.54	1.10	1.24	1.66	2.43	0.98	2.13	1.13	1.32	2.88	3.71	0.33	0.41	0.60	0.37	0.55	0.94	1.14	0.95	2.34

384	p1C14	0.44	3.47	0.52	0.64	0.96	0.89	4.73	3.06	1.92	0.59	0.69	1.91	1.10	2.27	1.64	2.85	2.68	2.62	7.82	2.55	1.95	1.59	1.62	0.47	0.44	0.39	0.35	0.45	1.13	0.50	0.76	0.79
386	p1C15	1.40	0.82	0.84	0.50	0.65	0.50	2.02	5.84	2.20	2.00	1.39	1.64	0.37	0.42	0.46	0.58	0.66	0.61	0.97	0.72	0.47	0.73	0.78	30.7	28.4	10.3	2.22	5.85	6.44	1.82	1.79	1.18
388	p1C16	2.84	1.76	2.30	0.95	1.80	1.11	0.95	5.76	5.09	1.57	1.27	0.98	0.66	0.68	1.25	1.79	1.76	0.94	25.0	14.8	0.37	0.41	0.59	0.42	0.61	0.33	0.35	0.67	1.30	0.63	1.28	0.97
390	p1C19	2.33	1.63	1.78	0.58	0.90	0.67	0.73	1.35	1.04	0.98	0.97	0.98	0.33	0.33	13.7	17.4	26.3	4.16	5.72	3.15	1.55	1.72	4.66	0.72	0.74	0.80	0.76	1.04	1.09	0.50	0.61	0.65
392	p1C20	0.68	1.08	1.96	1.56	1.12	1.04	2.89	4.45	4.18	0.71	1.25	1.76	0.31	0.47	0.27	0.48	0.55	0.81	1.58	1.69	0.51	0.82	1.21	0.51	0.37	1.45	1.10	3.34	4.67	0.67	2.21	1.87
396	p1P5	3.26	1.18	1.71	70.5	33.8	21.0	1.93	6.73	7.47	1.84	2.20	1.55	0.75	0.67	1.12	0.57	0.53	0.17	0.23	0.12	0.94	0.49	0.27	0.53	0.47	0.33	6.09	2.15	4.70	0.79	0.78	0.59
398	p2L23	0.99	0.66	0.57	63.1	35.3	56.2	245	289	339	0.47	0.50	0.42	0.47	1.04	0.30	0.32	0.29	2.63	1.23	1.15	0.22	0.31	0.34	0.77	0.70	0.55	28.3	15.9	28.5	2.59	2.39	2.30
402	p1K9	1.09	0.61	1.08	1.23	0.55	1.15	1.42	2.46	2.34	2.23	1.48	1.83	0.01	0.01	0.18	0.24	0.25	1.96	1.86	1.27	0.26	0.44	0.57	0.01	0.00	0.00	0.82	1.72	0.90	1.97	2.33	1.62
404	p1K23	1.91	1.08	1.43	0.91	0.60	0.75	1.67	1.98	1.51	2.01	1.52	1.89	0.72	0.72	0.18	0.18	0.10	3.36	1.73	1.21	0.09	0.11	0.13	0.88	0.47	0.13	2.17	2.19	3.26	1.01	0.95	0.92
406	p1K15	2.31	1.24	1.63	1.23	2.38	1.19	0.89	1.09	0.82	1.11	0.96	0.84	2.57	2.98	13.2	14.6	17.8	0.43	0.63	0.43	0.68	1.01	4.72	0.43	0.50	0.26	0.28	0.31	0.36	1.25	1.49	1.08
408	p1K8	1.32	0.86	0.76	0.97	1.35	1.29	1.08	1.98	1.56	0.98	0.71	0.72	0.94	1.46	3.51	1.11	0.61	0.32	0.73	0.26	3.47	2.77	0.84	0.56	0.76	0.60	0.59	0.97	0.81	1.32	1.83	1.20
410	p1M24	1.71	0.94	1.37	1.88	2.31	1.49	1.24	1.49	1.29	0.81	0.74	1.10	0.42	0.75	1.22	0.69	1.28	0.71	1.79	0.89	0.55	1.55	1.03	0.78	0.83	0.70	0.47	0.89	0.90	1.71	3.29	1.88
412	p1K7	2.24	0.79	0.56	1.84	0.69	0.58	2.40	4.45	2.78	2.16	1.57	1.05	0.62	0.51	0.46	0.21	0.13	1.44	0.62	0.43	0.32	0.37	0.31	2.24	1.03	0.61	3.53	2.35	1.35	1.53	1.43	0.63
414	p1K16	1.23	0.58	0.37	1.14	0.64	0.49	2.19	2.31	1.76	1.05	0.69	0.64	0.48	0.50	0.41	0.19	0.15	0.96	1.55	1.43	0.20	0.16	0.11	3.56	2.37	1.22	2.65	3.65	1.49	1.13	1.07	0.48
416	p1K18	3.59	1.63	1.92	1.02	2.11	1.23	1.07	1.33	0.92	2.28	1.71	1.51	0.89	0.76	1.94	1.43	1.92	0.11	0.10	0.12	0.57	1.20	1.35	0.58	0.72	0.43	0.54	0.64	0.61	0.91	1.02	0.91
418	p1N1	0.83	0.42	0.54	1.45	0.67	0.85	1.38	1.52	1.32	1.28	0.96	1.03	0.24	0.36	0.24	0.13	0.16	0.86	0.97	0.88	0.14	0.28	0.30	2.03	1.33	1.80	1.75	2.03	1.64	1.84	1.86	1.24

420	p1K22	1.82	1.13	0.74	0.90	0.73	0.51	2.20	2.31	1.76	1.27	0.91	1.03	0.55	0.61	0.37	0.26	0.24	1.30	1.51	0.67	0.20	0.23	0.21	1.45	1.17	0.40	1.52	1.16	1.00	1.06	1.39	0.74
422	p1K14	0.87	0.94	1.02	1.06	0.96	0.95	1.39	1.74	1.21	0.96	1.02	1.08	1.14	1.56	1.10	1.30	1.52	0.63	0.53	0.42	1.80	1.69	1.89	0.74	1.04	0.66	0.65	0.58	0.64	1.02	0.93	1.04
424	p1K13	1.70	0.82	1.59	1.14	0.76	1.10	6.27	4.09	4.13	3.03	2.53	2.33	0.43	0.46	1.33	0.75	0.84	0.31	0.53	0.38	0.61	0.88	0.66	0.70	0.46	0.45	1.80	1.96	1.24	1.79	1.29	0.94
426	p1J20	1.07	0.84	1.33	0.87	1.05	0.29	1.50	1.73	1.35	1.30	1.44	0.95	1.27	1.84	0.87	0.58	0.58	1.10	0.48	0.15	0.75	0.87	1.10	1.47	0.97	0.73	1.16	0.80	0.63	1.40	1.13	0.79
428	p1J22	2.11	2.14	1.90	1.00	1.69	1.41	0.74	0.86	0.59	1.95	1.68	1.34	2.55	2.75	2.32	2.40	2.83	0.16	0.10	0.10	2.06	2.23	1.40	0.40	0.65	0.27	0.23	0.19	0.29	1.01	0.98	0.91
430	p1K1	1.09	1.27	0.65	1.74	0.79	0.67	1.18	1.46	1.08	0.47	0.46	0.56	1.06	1.93	1.11	0.70	1.03	1.03	4.33	3.72	0.40	0.66	0.58	1.23	1.33	1.38	1.30	0.74	1.01	0.88	0.96	0.65
432	p1K3	1.18	1.11	0.83	1.19	1.95	1.20	0.61	0.78	0.47	0.97	0.83	0.75	1.00	1.54	55.5	32.9	34.3	0.42	0.50	0.30	10.4	12.4	16.5	0.88	0.80	0.89	0.58	0.48	0.66	1.41	1.39	1.65
434	p1J19	1.62	1.24	0.85	1.63	2.03	2.49	0.50	0.73	0.54	0.76	0.58	0.68	0.99	1.77	7.90	4.54	4.19	0.76	0.95	0.62	21.6	18.8	17.4	0.69	0.73	0.62	0.41	0.38	0.60	2.16	2.58	1.92
434	p1K2	1.06	0.80	0.92	1.67	1.95	1.52	0.41	0.82	0.39	0.94	0.87	0.83	0.78	0.83	10.1	5.86	4.93	1.00	0.97	0.59	25.5	24.5	22.4	0.76	0.98	0.62	0.42	0.44	0.62	2.44	2.95	1.93
436	p1K5	0.93	0.43	0.81	0.93	0.54	0.54	1.58	1.27	1.28	1.24	0.89	1.19	0.23	0.31	0.17	0.18	0.17	1.11	1.34	0.98	0.19	0.27	0.38	2.68	2.64	2.54	1.49	1.76	1.46	1.27	1.08	1.18
438	p1J17	0.93	0.72	0.95	1.11	1.25	0.49	0.93	0.65	0.39	1.09	0.58	0.45	0.95	1.11	2.10	2.41	1.70	0.83	0.35	0.11	1.31	1.52	2.62	1.52	0.65	0.40	1.30	0.54	0.36	2.26	1.42	0.85
440	p1J18	0.31	0.18	0.36	1.10	0.53	0.26	1.04	0.44	0.24	0.94	0.43	0.44	1.35	1.30	2.83	4.02	2.20	1.47	0.49	0.16	1.57	2.24	3.13	2.02	0.64	0.41	1.76	0.77	0.52	3.09	2.01	0.82
442	p1J15	0.55	0.66	1.26	0.99	0.68	0.22	1.68	1.41	1.25	1.75	1.55	1.24	1.47	2.14	0.69	0.65	0.20	0.95	0.45	0.13	0.65	0.68	0.73	1.78	1.03	0.67	1.72	0.99	0.75	1.91	1.36	0.95
444	p1K4	0.41	0.21	0.23	1.61	1.07	1.06	1.38	1.29	0.96	0.90	0.65	0.68	0.67	1.07	0.66	0.35	0.30	2.98	2.04	0.80	0.10	0.11	0.18	1.14	0.83	1.71	1.43	1.81	2.39	1.96	3.28	3.23
446	p2A14	2.61	2.13	1.22	0.87	1.15	1.12	0.95	1.36	0.83	2.04	1.59	1.21	1.97	2.23	1.18	1.21	1.44	0.47	0.31	0.22	1.74	1.39	1.04	0.67	0.86	0.43	0.62	0.68	0.54	0.64	0.71	0.59

448	p1J23	0.96	0.57	0.50	1.05	1.18	1.38	0.73	1.01	0.70	0.54	0.42	0.54	0.94	1.33	10.5	5.19	2.21	21.1	25.3	28.6	9.69	14.3	8.43	0.79	0.84	0.66	0.40	0.55	0.42	1.10	1.12	0.93
450	p1J21	0.75	0.68	0.96	1.16	1.09	0.52	1.61	1.35	1.35	1.15	0.96	0.79	0.98	1.60	0.56	0.66	0.35	1.18	0.82	0.38	0.54	0.65	0.63	1.67	1.15	0.80	1.28	0.99	0.87	1.90	1.45	1.16
452	p1J24	1.62	0.56	0.84	1.98	1.51	0.96	1.56	2.04	1.37	1.05	0.81	0.52	0.56	0.62	0.30	0.27	0.28	1.05	0.90	0.60	0.21	0.24	0.25	2.13	1.32	0.57	1.95	1.15	0.76	2.32	2.16	1.11
454	p1J16	3.62	1.55	2.31	1.38	2.76	1.29	2.85	4.20	3.66	1.65	1.84	1.78	0.53	0.76	1.04	0.62	0.68	0.33	0.38	0.23	0.49	0.53	0.53	0.88	0.70	0.57	1.02	0.94	0.72	1.49	1.60	1.29
456	p1J2	0.77	0.76	0.37	1.57	0.75	0.51	2.52	3.12	2.29	0.38	0.28	0.45	0.72	1.14	0.63	0.30	0.43	1.28	1.69	0.86	0.57	0.52	0.42	2.02	2.14	1.12	1.16	2.18	1.75	1.62	1.56	0.75
458	p1J9	0.73	0.45	0.38	1.22	1.38	1.06	2.10	1.00	0.87	0.38	0.34	0.34	0.71	1.18	3.04	3.92	3.04	0.94	1.07	0.55	9.00	11.36	7.81	1.90	1.06	0.76	0.36	0.26	0.25	0.82	0.75	1.10
460	p1J10	0.86	0.48	0.72	1.18	1.10	0.85	1.70	2.20	1.82	1.20	0.92	1.08	0.26	0.32	0.62	0.48	0.39	0.78	0.97	0.64	1.48	1.10	0.84	1.34	0.95	0.78	1.34	1.56	1.29	1.40	1.82	1.25
462	p1J1	0.97	0.70	0.68	1.41	1.09	0.87	3.29	3.09	2.75	0.63	0.45	0.51	0.63	0.86	0.55	0.46	0.59	0.97	1.90	0.78	0.41	0.40	0.47	7.28	5.01	2.77	1.96	3.36	2.23	1.53	1.81	1.16
464	p1J5	2.26	0.94	0.74	56.2	22.5	16.6	1.12	2.88	2.58	1.22	0.79	0.83	1.06	1.15	3.02	1.07	1.06	0.53	0.98	0.47	0.61	0.97	0.55	0.50	0.63	0.48	1.09	0.78	1.24	1.19	0.95	1.04
466	p1J11	0.95	0.57	0.30	0.71	0.51	0.60	0.95	0.70	0.70	0.37	0.22	0.18	0.86	1.20	1.77	0.97	1.15	1.60	2.41	1.21	0.99	1.52	0.88	1.11	1.00	0.37	2.03	2.53	2.90	3.37	2.53	2.45
468	p1J8	1.77	0.67	1.20	1.73	0.98	0.86	1.16	1.88	1.28	1.70	1.59	1.57	0.28	0.34	0.11	0.08	0.06	0.55	0.87	1.22	0.08	0.14	0.14	3.15	2.84	1.11	2.76	2.77	2.38	0.89	0.97	0.64
470	p1I20	2.84	1.42	1.67	1.17	2.14	1.41	0.99	1.46	1.03	0.79	0.72	0.92	0.74	0.90	7.59	2.80	2.20	0.34	0.55	0.24	1.76	1.84	1.12	0.46	0.55	0.44	0.28	0.38	0.44	1.26	2.02	1.06
472	p1J3	0.70	0.39	0.64	1.75	1.28	1.04	1.24	1.61	1.81	0.94	0.69	0.85	0.33	0.52	0.53	0.51	0.85	0.99	3.72	2.08	0.28	0.37	0.41	2.76	3.52	1.80	1.06	1.71	1.56	2.20	2.39	1.64
474	p1J12	7.58	4.86	4.02	0.90	4.17	1.83	0.84	1.06	0.78	1.06	0.88	0.90	1.01	0.94	3.33	4.41	4.72	0.37	0.30	0.29	0.85	0.66	1.25	2.71	1.82	1.00	0.60	1.22	0.89	0.99	1.49	0.83
476	p1I23	1.35	0.87	0.71	1.36	2.00	1.43	0.71	1.03	0.70	0.80	1.12	1.23	0.41	0.84	2.67	1.69	3.03	0.46	0.77	0.41	12.0	15.2	26.0	0.53	0.59	0.38	0.51	0.49	0.49	1.33	1.69	1.01

478	p1J7	1.54	0.65	0.90	1.22	1.15	0.65	1.34	1.20	1.06	1.50	1.07	0.97	0.26	0.31	0.40	0.28	0.33	0.47	0.69	0.28	0.23	0.26	0.30	2.35	1.61	1.05	1.14	1.62	1.05	3.03	2.25	1.61
480	p1I21	1.76	0.96	0.81	31.8	11.1	9.16	1.33	2.83	2.80	1.14	0.85	0.92	1.16	1.74	1.19	0.42	0.57	0.43	0.63	0.30	0.53	0.65	0.44	0.63	0.78	0.60	1.19	1.06	1.14	1.24	1.12	1.06
482	p1I19	1.15	0.74	0.71	11.42	5.80	5.51	0.83	2.40	2.91	0.82	0.66	0.94	6.67	8.50	0.41	0.34	0.44	2.15	1.21	1.23	0.82	1.84	1.35	0.32	0.32	0.24	7.08	6.21	1.83	0.66	0.80	0.53
484	p1J4	1.92	0.80	0.63	2.15	0.85	0.63	3.22	7.24	4.65	2.55	1.77	1.05	0.91	1.08	0.13	0.07	0.08	0.55	0.55	0.31	0.13	0.18	0.18	6.44	2.85	3.01	5.06	4.87	1.88	1.09	1.73	0.77
486	p1I24	3.43	1.85	1.70	20.5	11.56	9.6	0.51	1.70	1.90	1.00	0.84	0.98	2.75	3.80	0.67	0.77	1.14	0.58	0.32	0.30	0.22	0.35	0.28	0.40	0.49	0.37	8.92	9.81	2.94	1.02	1.14	0.72
488	p1I18	1.27	0.84	0.91	6.72	5.15	4.95	1.01	1.60	1.28	0.81	0.81	0.67	0.80	1.49	0.85	0.58	0.79	0.65	0.75	0.81	1.44	2.23	1.77	0.99	1.12	0.98	0.69	1.24	1.55	2.56	2.75	1.99

TABLE 13 Response of Novel genes to Hypoxia

CLONE ID	GENE NAME	SEQ Ids	HIGHEST FOLD CHANGE IN HYPOXIA (hr hypoxia + cell type)
p1F6	Hypothetical protein hqp0376 protein	337/338	67.4 (18hr monocyte)
p1E7	Novel metallothionein	83/84	37.9 (18hr monocyte)
p1D4	Hypothetical protein FLJ20500	25/26	23.8 (18hr monocyte)
p1D1	Hypothetical protein FLJ10134	23/24	14.75 (18hr neuro)
p1H13	EST	193/194	12.5 DOWN (18hr mam epithelial)
p1F13	Hypothetical protein FLJ13356 fis, clone PLACE1000050	343/344	9.26 (18hr monocyte)
p1H6	EST	191/192	8.42 (6hr cardiomyocyte)
p1H17	EST	171/172	8.33 DOWN (18hr mam epithelial)
p1E14	unknown mRNA (schizophrenia-linked)	97/98	7.79 (6hr mam epithelial)
p1P14	Hypothetical protein KIAA1745	91/92	7.32 (18hr renal epithelial)
p1H19	EST	195/196	7.14 DOWN (18hr cardiomyocyte)
p1D11	EST	135/136	6.90 (6hr mam epithelial)
p1D17	Hypothetical protein KIAA1745	91/92	6.74 (6hr cardiomyocyte)
p1F9	Hypothetical protein KIAA0742	19/20	6.64 (18hr monocyte)
p1D2	Hypothetical protein FLJ10134	23/24	6.62 (18 hr neuro)
p1H21	Hypothetical protein FLJ13511	163/164	6.61 (18hr monocyte)
p1D16	cDNA FLJ20308 fis, clone HEP07264	33/34	6.29 (18hr neuroblastoma)
p1D12	Hypothetical protein KIAA1376	29/30	5.98 (6hr cardiomyocyte)
p1H3	EST	215/216	5.88 DOWN (18hr mam epithelial)
p1H10	EST	189/190	4.98 (6hr cardiomyocyte)
p1D18	cDNA FLJ13443 fis, clone PLACE1002853	127/128	4.84 (6hr cardiomyocyte)
p1H4	EST	213/214	4.76 DOWN (18hr mam epithelial)
p1I4	Hypothetical protein HSPC196	53/54	4.54 DOWN (18he mam epithelial)
p1G20	cDNA YO23H03	203/204	4.17 DOWN (18hr cardiomyocyte)

p1I15	Hypothetical protein CGI-117	47/48	4.17 DOWN (18hr adipocyte)
p1F8	Hypothetical protein KIAA0914	09-Oct	3.88 (6hr mam epithelial)
p1H20	EST	179/180	3.84 DOWN (18hr cardiomyocyte)
p1I22	Hypothetical protein KIAA1429	37/38	3.70 DOWN (18hr mam epithelial)
p1E16	cDNA DKFZp586E1624	65/66	3.56 (6hr endothelial)
p1H15	EST	177/178	3.45 DOWN (18hr mam epithelial)
p1F5	Hypothetical protein FLJ20281	11-Dec	3.36 (6hr mam epithelial)
p1E1	EST	123/124	3.24 (6hr cardiomyocyte)
p1H7	EST	175/176	3.13 DOWN (18hr mam epithelial)
p1D19	EST	143/144	2.98 (6hr cardiomyocyte)
p1F21	cDNA FLJ14342 fis, clone THYRO1000569	17/18	2.92 (18hr monocyte)
p1H12	EST	173/174	2.84 (6hr hepatocyte)
p1F2	Hypothetical protein FLJ20037	03-Apr	2.84 (18hr monocyte)
p1H23	cDNA FLJ21094 fis, clone CAS03807	187/188	2.78 DOWN (18hr neuroblastoma)
p1I13	Hypothetical protein FLJ11100	43/44	2.78 DOWN (18hr adipocyte)
p1D20	Hypothetical protein KIAA1125	139/140	2.73 (6hr renal epithelial)
p1E11	EST	109/110	2.73 (6hr hepatocyte)
p1D9	Hypothetical protein DKFZP564D116	27/28	2.71 (6hr adipocyte)
p1H5	Hypothetical protein FLJ22690	205/206	2.55 (6hr cardiomyocyte)
p1D24	EST	117/118	2.55 (18hr renal epithelial)
p1E12	Hypothetical protein DKFZP434E1723	69/70	2.49 (6hr mam epithelial)
p1F10	Hypothetical protein DKFZp434P0116	05-Jun	2.45 (6hr mam epithelial)
p1G22	EST	197/198	2.38 DOWN (18hr mam epithelial)
p1E4	EST	125/126	2.31 (18hr monocyte)
p1I3	Hypothetical protein FLJ11656	153/154	2.27 DOWN (18hr adipocyte)
p1F12	EST	01-Feb	2.27 (6hr hepatocyte)
p1G18	Mitochondrion sequence	211/212	2.17 (18hr neuroblastoma)
p1E23	cDNA FLJ14041 fis, clone HEMBA1005780	111/112	2.16 (18hr monocyte)
p1E9	novel PI-3-kinase adapter	79/80	2.15 (18hr monocyte)
p1G7	EST	281/282	2.14 (18hr monocyte)
p1J13	Hypothetical nuclear factor SBBI22	35/36	2.13 DOWN (18hr adipocyte)
p1E19	EST	105/106	2.13 (6hr hepatocyte)

p1E15	cDNA YI27F12	107/108	2.12 (18hr monocyte)
p1F23	Hypothetical protein LOC51014	289/290	2.10 (6hr endothelial)
p2A14	EST	445/446	2.08 DOWN (18hr mam epithelial)
p1I17	Hypothetical protein FLJ20644	45/46	2.08 (6hr endothelial)
p1E8	cDNA: FLJ22249 fis, clone HRC02674	61/62	2.07 (6hr endothelial)
p1H1	Hypothetical protein FLJ10826	201/202	2.07 (18hr endothelial)
p1I10	EST	155/156	2.04 DOWN (18hr mam epithelial)
p1I5	Hypothetical protein FLJ10815	41/42	2.04 (6hr cardiomyocyte)
p1E20	Hypothetical protein FLJ20421	99/100	2.01 (6hr renal epithelial)
p1C23	cDNA FLJ12832 fis, clone NT2RP2003137	133/134	2.01 (6hr monocyte)
p1J16	cDNA: FLJ23019 fis, clone LNG00916	453/454	2.00 (6hr cardiomyocyte)
p1I12	Hypothetical protein MGC4549	151/152	1.96 DOWN (18hr mam epithelial)
p1F1	EST	81/82	1.96 (18hr monocyte)
p1E22	cDNA FLJ13618 fis, clone PLACE1010925	161/162	1.95 (6hr monocyte)
p1D21	Hypothetical protein FLJ22622	129/130	1.95 (6hr cardiomyocyte)
p1F19	Hypothetical protein KIAA0212	07-Aug	1.95 (18hr monocyte)
p1F18	Hypothetical protein KIAA0876	13/14	1.92 DOWN (6hr adipocyte)
p1H9	EST	185/186	1.91 (18hr monocyte)
p1F11	Hypothetical protein LOC51754	323/324	1.90 (6hr macrophage)
p1I2	cDNA FLJ11302 fis, clone PLACE1009971	149/150	1.88 (18hr monocyte)
p1G21	EST	199/200	1.85 DOWN (6hr macrophage)
p2A24	EST	101/102	1.85 DOWN (18hr adipocyte)
p1E13	Hypothetical protein PRO0823	21/22	1.85 DOWN (18hr adipocyte)
p1E10	cDNA FLJ11041 fis, clone PLACE1004405	71/72	1.84 (6hr endothelial)
p1I14	cDNA DKFZp564D016	147/148	1.80 (6hr monocyte)
p1I6	Hypothetical protein FLJ10206	39/40	1.78 (6hr mam epithelial)
p1F3	Hypothetical protein LOC94951	333/334	1.75 (6hr renal epithelial)
p1I16	Hypothetical protein KIAA1668	57/58	1.69 (6hr mam epithelial)
p1H16	EST	183/184	1.64 (18hr renal epithelial)
p1E17	cDNA FLJ31668 fis, clone NT2RI2004916	103/104	1.63 (6hr cardiomyocyte)
p1I8	hypothetical protein FLJ11296	55/56	1.61 (18hr macrophage)
p1H14	EST	167/168	1.45 (6hr renal epithelial)

TABLE 14 Response of Novel genes to Hypoxia

CLONE ID	GENE NAME	SEQ Ids	HIGHEST FOLD CHANGE IN HYPOXIA (hr hypoxia + cell type)
p1D6	ERO1 (S. cerevisiae)-like	67/68	11.30 (18hr fibroblast)
p1D10	Insulin induced protein 2	75/76	8.14 (18hr renal epithelial)
p1H2	Fatty acid binding protein 5	209/210	7.14 DOWN (18hr neuroblastoma)
p1H18	Ubiquitin specific protease 7	157/158	7.14 DOWN (18hr mam epithelial)
p1D22	MAX-interacting protein 1	119/120	6.68 (18hr renal epithelial)
p1C24	SLC25A19	93/94	6.13 (18hr macrophage)
p1E3	CYP1B1	137/138	5.88 DOWN (18hr renal epithelial)
p1G19	Mitochondrion sequence	207/208	5.88 DOWN (18hr mam epithelial)
p1D14	C1orf12	89/90	5.68 (6hr cardiomyocyte)
p1H8	ABL	181/182	4.76 DOWN (18hr cardiomyocyte)
p1E6	EGL nine (C.elegans) homolog 3	85/86	4.63 (18hr mam epithelial)
p1D13	Adenylate kinase 3	77/78	4.58 (6hr cardiomyocyte)
p1H24	Nucleolar phosphoprotein Nopp34	159/160	4.40 (6hr cardiomyocyte)
p1D15	TRIP-Br2	31/32	4.09 (18hr renal epithelial)
p1F7	Spectrin, beta, non-erythrocytic 1	15/16	2.65 (6hr endothelial)
p1E5	Hepcidin antimicrobial peptide	141/142	2.59 (6hr macrophage)
p1C22	CD84-H1	131/132	2.58 (6hr cardiomyocyte)
p1E2	Mannosidase, alpha, class 1A, member 1	121/122	2.56 DOWN (18hr neuroblastoma)
p1C21	Tubulin, beta, 4	73/74	2.51 (6hr cardiomyocyte)
p1D3	Serine carboxypeptidase 1	95/96	2.49 (18hr fibroblast)
p1H11	Carboxypeptidase M	169/170	2.18 (18hr monocyte)
p1E18	Plexin C1	63/64	2.15 (6hr hepatocyte)
p2B1	PRAME	87/88	2.13 DOWN (18hr fibroblast)
p1E21	Glutamate-cysteine ligase, modifier subunit	113/114	2.04 (6hr hepatocyte)

p1I11	SECIS binding protein 2	59/60	2.00 DOWN (18hr endothelial)
p1I1	Ribosomal RNA intergenic spacer	165/166	1.92 DOWN (18hr neuroblastoma)
p1I7	Uridine 5' monophosphate hydrolase 1	49/50	1.77 (18hr monocyte)
p1D23	PTEN	115/116	1.74 (6hr renal epithelial)
p1D5	ERO1 (S. cerevisiae)-like	67/68	1.72 (6hr mam epithelial)
p2A15	Sialyltransferase	145/146	1.61 DOWN (6hr monocyte)

TABLE 15 Genes with increased expression by macrophage activation

			mRNA EXPRESSION (experimental condition)					
Clone	Seq ID	Gene Name	#1	#2	#3	#4	#5	#6
p1K8	407/408	SCYA4	0.82	0.40	1.15	0.38	91.4	68.4
p1B16	251/252	Interleukin 8	0.75	1.13	0.47	0.41	42.8	28.1
p1B15	251/252	Interleukin 8	0.85	1.12	0.44	0.37	47.4	22.5
p1I21	479/480	SCYA8	0.54	0.18	1.15	0.32	19.6	12.2
p1I20	469/470	SCYA3L	0.92	0.41	1.00	0.30	29.4	22.8
p1N17	237/238	COX-2	0.90	1.00	0.84	0.84	18.9	20.3
p1J16	453/454	cDNA: FLJ23019 fis, clone LNG00916	0.92	0.66	0.91	1.15	14.4	14.9
p1I7	49/50	Uridine 5' monophosphate hydrolase 1	1.13	0.57	0.99	0.52	17.6	23.7
p1B14	251/252	Interleukin 8	0.71	1.20	0.51	0.47	10.1	21.4
p1E10	71/72	cDNA FLJ11041 fis, clone PLACE1004405	0.66	0.74	1.15	0.81	8.30	12.1
p2L23	397/398	endothelin 1	1.02	0.62	0.74	0.50	11.4	10.1
p1D19	143/144	EST	0.63	0.52	1.00	1.16	5.46	4.73
p1K3	431/432	Pleckstrin	1.14	0.70	0.73	0.54	6.49	2.34
p1C9	373/374	RAB-8b protein	0.95	0.81	0.77	0.94	5.11	4.53
p1I24	485/486	GRO1	0.90	0.72	0.78	1.04	4.69	2.96
p1G3	317/318	B-cell translocation gene 1	0.70	1.00	0.57	1.14	3.51	3.79
p1B1	243/244	Metallothionein 1G	0.51	1.00	0.66	1.85	2.50	3.83
p1J11	465/466	Fatty-acid-Coenzyme A ligase, long-chain 2	0.69	0.51	1.36	0.91	3.07	2.97
p1F17	329/330	P8 protein (candidate of metastasis 1)	0.26	1.78	0.16	0.88	1.16	2.59
p1F4	339/340	CYP1	0.60	1.04	0.77	1.15	2.52	4.22
p1D10	75/76	Insulin induced protein 2	0.49	1.00	0.48	1.23	1.63	4.96
p1E7	83/84	Novel metallothionein	0.49	1.26	0.70	1.11	1.32	2.89
p1D24	117/118	EST	0.58	0.71	1.00	1.32	1.56	2.59
p1I19	481/482	GRO2	0.99	1.00	0.69	0.55	2.65	2.29
p1E22	161/162	cDNA FLJ13618 fis, clone PLACE1010925	1.19	0.77	0.85	0.51	3.09	2.41
p1F6	337/338	Hypothetical protein hqp0376	0.44	1.08	0.47	1.06	1.11	2.47
p1J7	477/478	Sjogren syndrome antigen B	1.06	0.74	0.85	0.63	2.65	2.95
p1B19	235/236	plasminogen activator inhibitor, type 1	0.59	1.29	0.45	1.17	1.46	3.46
p1F24	297/298	Glia-derived nexin	0.65	0.68	0.99	1.10	1.62	1.80
p1P5	395/396	SCYA2	0.94	0.13	3.81	0.42	2.27	1.00
p1A22	263/264	Adenylate kinase 3	0.57	1.30	0.58	1.64	1.34	2.74
p1A23	265/266	Metallothionein 2A	0.55	0.89	0.95	1.01	1.23	4.38
p1A24	239/240	Metallothionein 1H	0.50	0.84	1.03	1.02	1.08	1.60

p1P3	221/222	PDGFB	0.47	2.06	0.31	1.99	1.00	1.49
p1A7	345/346	SLC31A2	1.10	0.92	0.84	0.93	2.30	2.32
p1P14	91/92/92a	Semaphorin 4b	0.47	2.52	0.95	4.04	0.96	3.88

Legend

mRNA expression values in the 6 experimental conditions (#1 no cytokines/ normoxia, #2 no cytokines/ hypoxia, #3 IL-10/ normoxia, #4 IL-10/ hypoxia, #5 LPS/IFN/ normoxia, #6 LPS/IFN/ hypoxia) are shown as values referenced to the median value of that gene throughout all 6 experimental conditions.

TABLE 16. Genes down-regulated by macrophage activation

			mRNA EXPRESSION (experimental condition)					
Clone	Seq ID	Gene Name	#1	#2	#3	#4	#5	#6
p1H13	193/194	EST	1.35	1.41	1.00	0.91	0.44	0.68
p1E4	125/126	EST	1.22	1.13	1.09	0.96	0.40	0.40
p1G7	281/282	EST	1.30	1.44	1.01	1.51	0.54	0.82
p1E1	123/124	EST	1.21	1.64	0.94	1.35	0.51	0.63
p1D18	127/128	cDNA FLJ13443 fis, clone PLACE1002853	1.61	2.60	0.57	1.33	0.26	0.24
p1I2	149/150	cDNA FLJ11302 fis, clone PLACE1009971	2.39	1.23	1.07	0.54	0.45	0.43
p1G20	203/204	cDNA YO23H03	1.45	0.73	1.60	1.12	0.57	0.44
p1D21	129/130	Hypothetical protein FLJ22622	1.41	1.72	0.82	1.26	0.14	0.14
p1F8	9/10	Hypothetical protein KIAA0914	1.34	4.14	0.77	2.74	0.13	0.25
p1D16	33/34	Hypothetical protein FLJ20308	1.31	2.36	1.00	1.59	0.29	0.67
p1F3	333/334	Hypothetical protein XP_017131	1.63	2.21	0.92	1.00	0.42	0.42
p1D12	29/30	Hypothetical protein KIAA1376	0.89	2.62	0.79	2.07	0.28	2.61
p1I4	53/54	Hypothetical protein HSPC196	1.95	1.06	1.20	0.57	0.63	0.28
p1D9	27/28	Hypothetical protein DKFZP564D116	1.63	0.94	1.25	0.96	0.55	0.85
p1F9	19/20	Hypothetical protein KIAA0742	0.94	3.54	0.60	1.74	0.33	1.74
p1F11	323/324	Hypothetical protein LOC51754	1.67	1.91	1.00	0.86	0.60	0.59
p1I15	47/48	Hypothetical protein CGI-117	1.31	0.62	1.86	1.26	0.49	0.76
p1E13	21/22	Hypothetical protein PRO0823	1.15	0.93	1.15	1.08	0.44	0.24

p1F10	"5/6"	Hypothetical protein DKFZp434P0116	2.16	1.05	1.54	0.83	0.83	0.67
p1D1	23/24	Hypothetical protein FLJ10134	0.86	1.70	0.61	2.42	0.35	1.61
p1I5	41/42	Hypothetical protein FLJ10815	1.49	1.00	1.30	0.83	0.43	0.37
p1G13	293/294	ABCA1	1	1.05	1.22	1.07	0.43	0.46
p1B9	313/314	adipophilin	1.01	3.74	1.32	2.08	0.02	0.2
p1B7	313/314	adipophilin	1.57	3.44	0.77	1.17	0.21	0.45
p1B6	313/314	adipophilin	1.24	2.45	0.8	1.51	0.38	0.52
p1B8	313/314	adipophilin	1.11	1.87	1.01	1.04	0.55	0.59
p1K7	411/412	ATP-binding cassette E1	1.34	0.74	1.58	1.05	0.62	0.48
p1J23	447/448	Calgranulin A	1.21	0.94	3.35	2.8	0.58	0.86
p1K18	415/416	Colony-stimulating factor1	2.01	0.84	1.7	0.88	1	0.74
p1C2	331/332	CXCR4	1.01	3.76	0.46	1.47	0.08	1.13
p1C1	331/332	CXCR4	1.05	3.64	0.39	1.63	0.27	0.98
p1G12	321/322	Cyclin G2	0.85	2.17	0.6	1.29	0.28	1.33
p1F16	325/326	CYP1B1	1.37	0.96	1.67	0.66	0.64	0.48
p1C7	369/370	D123	1.69	1.33	1.1	0.7	0.65	0.83
p1G17	315/316	Early development regulator 2	0.97	2.47	1.12	2.24	0.29	0.85
p1I23	475/476	Ecotropic viral integration site 2A	1.39	1.25	1.11	1.72	0.18	0.22
p1A14	257/258	Enolase 1	0.99	3.22	1.19	2.46	0.13	0.37
p1A10	273/274	Enolase 2	1.17	5.28	0.59	3.77	0.49	1.08
p1D6	67/68	ERO1 (S. cerevisiae)-like	0.84	3.02	0.97	2.87	0.32	1.58
p1A11	253/254	GAPDH	1.21	2.41	0.93	1.31	0.32	0.81
p1A12	253/254	GAPDH	1.09	1.97	1	1.49	0.41	0.96
p1K22	419/420	GPR44	1.24	1.03	1.42	0.93	0.56	0.48
p1C18	269/270	Granulin	1.28	1.59	0.96	1.03	0.56	0.72
p1C17	269/270	Granulin	1.58	1.6	0.62	0.41	0.76	0.94
p1A15	249/250	Hexokinase-2	0.89	3.88	0.68	3.11	0.38	2.02
p1C13	381/382	Jk-recombination signal binding protein	1.11	1.18	1.43	1.98	0.32	0.73
p1A8	223/224	Lactate dehydrogenase A	0.7	2.25	1.4	1.44	0.26	1.32
p1A9	223/224	Lactate dehydrogenase A	0.77	1.85	1.15	1.68	0.32	1.19
p1G5	279/280	MAX-interacting protein 1	1.24	5.5	0.9	4.48	0.34	0.97
p1D22	119/120	MAX-interacting protein 1	1.2	3.86	0.52	3.44	0.37	0.91
p1G18	211/212	Mitochondrion sequence	1.27	1.12	1	1.31	0.57	0.77
p1K23	403/404	MYC	1.37	0.77	2.39	1.09	0.54	0.35
p1E20	99/100	Myo-inositol monophosphatase A3	1.12	1.28	1.02	0.99	0.48	0.61
p1B20	267/268	Osteopontin	1.13	1.58	0.99	1.52	0.1	0.4
p1F13	343/344	Papillomavirus regulatory factor PRF-1	0.98	5.02	0.44	6.79	0.09	2.43
p1A13	255/256	Phosphoglycerate kinase 1	1.04	2.45	1.23	1.83	0.2	0.9
p1G9	305/306	PI-3-kinase, catalytic, beta polypeptide	1.46	1.88	0.75	1.17	0.44	0.47

p1E18	63/64	Plexin C1	1.72	1.79	1	0.85	0.69	0.35
p1C11	377/378	polyubiquitin	1.13	1.79	0.79	1.14	0.5	0.84
p1B3	231/232	Proline 4-hydroxylase, alpha polypeptide I	0.94	1.38	1.03	1.58	0.43	0.89
p1B4	349/350	Proline 4-hydroxylase, alpha polypeptide II	0.9	1.46	1.05	1.41	0.44	1
p1B22	355/356	Protease, serine, 11	1.3	1.1	1.26	0.92	0.64	0.7
p1C10	375/376	Regulator of G-protein signalling 1	1.42	1.68	0.94	1.55	0.47	0.95
p1D3	95/96	Serine carboxypeptidase 1	1.22	1.07	1.07	1.09	0.33	0.88
p1F15	341/342	SHB adaptor protein	1.04	1.61	0.94	1.72	0.43	0.54
p1A5	311/312	SLC2A5	0.71	2.6	1.06	2.09	0.34	1.09
p1G4	307/308	SLC5A3	1.12	1.44	0.93	1.31	0.33	0.62
p1A20	261/262	Triosephosphate isomerase 1	0.97	2.06	1.09	2.24	0.17	0.66
p1D15	31/32	TRIP-Br2	1.16	1.4	1.1	1.25	0.47	0.46
p1K4	443/444	TSC-22	1.44	1	1.55	0.7	0.6	0.57

TABLE 17: Genes responsive to IL-10 (increased or decreased) but not affected significantly by LPS+IFN

			mRNA EXPRESSION (experimental condition)					
Clone	**Seq ID**	**Gene Name**	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**
p1H8	181/182	ABL	1.02	0.96	6.65	5.25	0.86	0.73
p1E15	107/108	cDNA YI27F12	0.48	0.77	1.69	2.45	0.78	1.44
p2A14	445/446	EST	1.06	0.74	2.78	3.09	1.06	0.82
p1H6	191/192	EST	1.01	0.84	2.47	2.05	0.93	0.83
p1E5	141/142	Hepcidin antimicrobial peptide	0.84	0.73	1.91	1.68	0.58	2.16
p1I12	151/152	Hypothetical protein MGC4549	1.07	0.67	2.34	2.53	1.11	0.74
p1D8	271/272	Hypoxia-inducible protein 2	0.65	1.00	1.51	1.89	0.71	2.05
p1K14	421/422	Keratin 6B	1.03	0.68	3.80	3.28	0.97	0.76
p1J22	427/428	Neutral sphingomyelinase (N-SMase) activation associated factor	0.94	0.79	5.59	3.52	0.91	1.29
p1G15	287/288	Phosphoglucomutase 1	0.82	1.20	1.83	1.90	0.61	1.05
p1A2	247/248	SLC2A3	0.37	3.31	1.00	3.32	0.49	2.63
p1A3	247/248	SLC2A3	0.39	2.45	1.00	2.65	0.20	1.50
p1K2	433/434	CFFM4	1.30	0.98	0.51	0.59	1.11	0.91
p1C4	363/364	FGF receptor activating protein 1	1.02	0.96	0.50	0.63	1.16	1.31

TABLE 18. Genes up-regulated in human tumors. Individual patients are denoted by the letters E,F,G,H and K.

			Ovary	Ovary	Ovary	Ovary	Ovary	Ovary	Ovary	Breast	Breast	Breast	Breast
			nor	tum	nor	tum	nor	tum	nor	tum	nor	tum	
Clone	Gene Name	SeqID	E	E	F	F	G	G	H	H	K	K	
p1H8	ABL	182	0.73	2.21	0.72	1.48	1.15	3.15	2.41	2.01	2.61	1.00	
p1B6	adipophilin	314	0.44	1.57	0.51	0.37	1.30	0.99	0.58	0.78	0.82	1.06	
p1A19	Aldolase C	260	0.27	1.00	0.74	1.06	0.40	1.49	0.62	0.47	0.49	2.18	
p1C2	CXCR4	332	0.29	0.91	1.03	1.41	2.43	2.80	2.71	0.95	1.81	0.59	
p1K1	Cyclophilin F	430	0.60	0.71	1.11	0.80	0.61	1.85	0.76	0.76	0.95	1.66	
p1E3	CYP1B1	138	0.32	0.06	0.45	1.47	1.05	0.16	1.00	0.38	1.20	0.16	
p1F16	CYP1B1	326	0.60	0.17	0.67	2.30	1.65	0.24	1.00	0.55	1.82	0.24	
p1C8	Dec1	372	3.93	0.66	1.85	1.10	1.37	0.56	0.94	0.53	0.87	5.55	
p1A14	Enolase 1	258	0.10	0.46	1.00	1.26	0.41	1.47	0.36	0.81	0.53	0.61	
p1A10	Enolase 2	274	0.63	0.64	1.48	3.23	0.80	2.67	0.96	0.92	0.92	0.52	
p1D6	ERO1 (S. cerevisiae)-like	68	0.30	1.61	0.73	1.32	0.77	0.31	0.66	0.42	1.10	1.02	
p1E19	EST	106	1.00	2.04	1.38	1.30	0.20	1.19	2.38	2.49	1.19	1.78	
p1H15	EST	178	0.22	0.46	0.71	1.07	0.82	2.57	2.62	1.22	3.66	1.07	
p1H16	EST	184	1.57	2.66	1.44	1.42	0.74	2.65	2.32	1.91	1.70	1.95	
p1H17	EST	172	0.65	2.20	1.00	1.62	0.95	2.64	2.95	2.32	2.98	0.93	
p1H20	EST	180	0.81	2.39	0.98	1.61	0.78	2.91	2.83	1.62	2.64	1.17	
p1H3	EST	216	0.10	0.38	0.78	1.14	0.88	2.49	2.26	1.18	3.08	0.89	
p1C4	FGF receptor activating protein 1	364	0.53	0.77	0.91	1.00	1.04	0.98	0.81	1.27	0.72	2.43	
p1A11	GAPDH	254	0.04	0.74	1.50	2.84	0.86	1.57	0.27	1.12	0.51	0.69	
p1C6	Glucose phosphate isomerase	368	0.21	0.86	1.59	2.26	0.65	1.94	0.47	0.79	0.61	1.00	
p1E21	Glutamate-cysteine ligase, modifier subunit	114	1.26	2.38	1.35	1.23	0.27	1.51	2.05	4.12	1.00	1.78	
p1C18	Granulin	270	0.44	0.91	1.00	0.83	0.71	0.88	0.60	0.61	0.73	2.72	

p1D21	Hypothetical protein FLJ22622	130	0.39	8.14	0.53	0.59	0.92	0.54	0.87	1.71	0.99	1.00
p1F11	Hypothetical protein LOC51754	324	0.53	0.77	1.01	0.97	0.61	1.88	1.54	1.10	0.85	0.70
p1A8	Lactate dehydrogenase A	224	0.24	1.07	1.09	1.00	0.70	0.40	0.52	0.50	0.66	0.92
p1K9	Lipocortin I	402	0.46	4.09	1.33	1.17	1.07	0.31	1.20	0.54	0.23	0.12
p1B1	Metallothionein 1G	244	1.21	0.60	3.14	1.49	1.00	2.04	0.46	1.54	0.27	0.51
p1K23	MYC	404	5.98	1.00	2.50	2.41	4.11	1.91	0.64	2.17	0.32	0.44
p1B20	Osteopontin	268	0.05	1.01	2.74	1.69	0.22	0.36	0.24	0.52	0.35	0.92
p1B21	Osteopontin	268	0.22	1.73	2.09	1.87	0.58	0.76	0.52	0.65	0.44	1.00
p1F17	P8 protein (candidate of metastasis 1)	330	1.18	0.17	1.27	1.47	0.55	1.79	1.00	0.97	0.92	1.33
p1C11	polyubiquitin	378	1.11	0.91	1.59	1.53	1.00	1.34	0.63	0.65	0.51	2.52
p2B1	PRAME	88	1.13	8.86	5.08	9.57	1.15	18.20	1.63	1.64	0.96	0.96
p1B5	Proline 4-hydroxylase, alpha polypeptide II	350	0.50	0.52	1.02	3.18	1.94	1.31	0.79	0.89	0.72	1.62
p1P14	Semaphorin 4b	92	0.80	4.26	0.86	1.01	1.22	1.88	1.33	1.73	1.48	2.26
p1A6	SLC2A5	312	0.52	5.07	0.89	2.12	1.48	0.68	1.21	0.55	1.37	0.87
p1J17	SLC6A1	438	0.11	0.39	0.85	1.42	0.80	3.56	3.08	1.17	4.08	1.00
p1J18	Synaptopodin	440	0.07	0.31	0.99	1.44	0.68	3.03	3.51	1.40	4.54	1.29
p1J15	TERA protein	442	0.68	2.14	0.80	1.47	1.00	2.36	2.97	2.74	3.14	0.97
p1G11	Tumor protein D52	302	0.20	1.49	0.51	1.07	0.83	1.00	1.66	1.82	1.90	2.62
p1H18	Ubiquitin specific protease 7	158	0.73	2.13	0.91	1.76	0.82	2.47	2.56	2.04	2.53	1.00
p1O20	VEGF	228	0.84	4.19	0.85	1.55	2.31	12.69	0.66	1.42	1.35	6.44

TABLE 19. Genes down-regulated in human tumors. Individual patients are denoted by the letters E,F,G,H and K.

			Ovary	Ovary	Ovary	Ovary	Ovary	Ovary	Breast	Breast	Breast	Breast
			nor	tum	nor	tum	nor	tum	nor	tum	nor	tum
Clone	Gene Name	SeqID	E	E	F	F	G	G	H	H	K	K
p1C3	Activin A receptor, type I	362	1.51	0.32	1.28	1.76	1.06	1.21	0.99	0.87	0.76	1.17
p1B9	adipophilin	314	0.67	0.20	0.78	0.50	1.53	2.45	0.61	0.83	0.68	0.85
p1K15	Alpha-2-macroglobulin	406	0.39	0.12	0.79	0.54	1.08	0.38	0.71	1.16	0.53	0.71
p1G3	B-cell translocation gene 1	318	2.01	0.64	1.15	1.13	1.69	0.58	1.39	1.13	1.66	0.78
p1F14	Butyrate response factor 1	328	2.85	0.94	1.86	2.31	1.46	0.79	1.36	1.00	0.86	1.09
p1J23	Calgranulin A	448	0.43	0.90	1.00	0.91	11.60	0.59	12.02	1.60	23.70	0.80
p1K2	CFFM4	434	0.47	0.29	1.30	1.06	2.32	0.31	1.00	0.49	0.79	1.52
p1J19	CFFM4	434	0.45	0.29	1.24	1.00	2.06	0.35	1.42	0.56	0.76	1.44
p2A23	Chitinase 3-like 2	284	0.66	0.78	0.48	0.61	4.18	0.74	1.36	2.22	0.91	2.01
p1N17	COX-2	238	0.73	1.21	0.51	0.72	2.31	0.57	0.80	0.61	0.56	0.54
p1C2	CXCR4	332	0.29	0.91	1.03	1.41	2.43	2.80	2.71	0.95	1.81	0.59
p1E3	CYP1B1	138	0.32	0.06	0.45	1.47	1.05	0.16	1.00	0.38	1.20	0.16
p1F16	CYP1B1	326	0.60	0.17	0.67	2.30	1.65	0.24	1.00	0.55	1.82	0.24
p1C8	Dec1	372	3.93	0.66	1.85	1.10	1.37	0.56	0.94	0.53	0.87	5.55
p1J10	DNCLI2	460	1.00	0.98	1.28	1.17	1.66	0.50	2.02	1.51	0.98	0.90
p1I23	Ecotropic viral integration site 2A	476	0.64	0.67	0.88	1.08	1.28	0.39	0.79	0.71	1.06	0.78
p1E4	EST	126	1.30	1.67	0.88	0.65	0.70	0.17	1.00	0.69	1.06	0.46
p1H19	EST	196	0.71	1.52	0.80	0.99	1.08	1.17	1.99	1.70	3.34	0.83
p1H4	EST	214	0.32	0.76	0.70	1.02	1.14	2.36	2.34	1.65	2.90	0.75
p1H3	EST	216	0.10	0.38	0.78	1.14	0.88	2.49	2.26	1.18	3.08	0.89
p1H15	EST	178	0.22	0.46	0.71	1.07	0.82	2.57	2.62	1.22	3.66	1.07
p1H17	EST	172	0.65	2.20	1.00	1.62	0.95	2.64	2.95	2.32	2.98	0.93
p1J11	Fatty-acid-Coenzyme A ligase, long-chain 2	466	0.83	0.23	0.68	1.38	1.05	0.44	1.42	0.85	1.00	1.00

p1F24	Glia-derived nexin	298	3.17	0.93	3.24	0.85	1.26	0.80	1.64	1.89	1.25	0.96
p1I24	GRO1	486	0.82	2.23	1.55	3.98	3.19	0.52	0.50	0.55	0.31	0.52
p1I19	GRO2	482	1.97	2.36	3.67	4.14	11.31	1.00	1.29	1.48	0.64	0.70
p1D1	Hypothetical protein FLJ10134	24	1.20	0.40	1.82	1.82	0.73	0.68	0.81	1.03	1.28	0.96
p1H21	Hypothetical protein FLJ13511	164	1.00	0.22	0.97	0.49	1.73	0.72	1.35	0.75	2.85	1.76
p1F2	Hypothetical protein FLJ20037	4	2.41	0.61	1.20	0.63	1.40	0.39	0.66	0.73	0.53	0.63
p1F23	Hypothetical protein LOC51014	290	1.27	0.85	0.90	1.03	1.82	0.55	0.90	0.92	0.84	0.94
p1E13	Hypothetical protein PRO0823	22	0.51	0.60	1.00	0.98	1.74	0.52	2.15	0.98	2.26	0.85
p1F3	Hypothetical protein XP_017131	334	2.75	0.91	1.26	1.39	1.42	0.61	1.67	1.96	1.13	1.02
p1B14	Interleukin 8	252	2.96	0.36	4.71	1.04	8.45	1.00	0.36	0.37	0.54	0.37
p1B16	Interleukin 8	252	3.16	0.50	7.46	1.82	16.49	3.29	1.00	0.42	0.66	0.36
p1B15	Interleukin 8	252	3.43	0.54	7.25	1.69	11.81	3.46	0.94	0.75	0.67	0.88
p1C13	Jk-recombination signal binding protein	382	1.94	0.55	1.48	1.17	0.54	0.50	0.54	0.80	0.51	0.57
p1K9	Lipocortin I	402	0.46	4.09	1.33	1.17	1.07	0.31	1.20	0.54	0.23	0.12
p1E2	Mannosidase, alpha, class 1A, member 1	122	0.87	0.40	1.00	0.88	2.02	0.50	1.51	0.67	1.54	1.63
p1A23	Metallothionein 2A	266	0.78	0.22	2.96	1.22	2.34	1.05	0.58	1.50	0.40	0.48
p1G19	Mitochondrion sequence	208	1.00	1.74	1.17	1.08	0.99	1.69	2.16	1.67	2.10	0.58
p1G18	Mitochondrion sequence	212	0.77	1.43	0.95	1.02	1.00	0.99	1.71	1.62	2.61	0.86
p1K23	MYC	404	5.98	1.00	2.50	2.41	4.11	1.91	0.64	2.17	0.32	0.44
p1J20	Neuro-oncological ventral antigen 1	426	0.54	1.61	1.00	1.50	1.24	2.65	3.08	2.55	2.77	0.83
p1F17	P8 protein (candidate of metastasis 1)	330	1.18	0.17	1.27	1.47	0.55	1.79	1.00	0.97	0.92	1.33
p1B19	plasminogen activator	236	3.22	0.44	2.15	1.84	7.67	2.48	0.97	0.56	0.88	0.86

	inhibitor, type 1												
p1B18	plasminogen activator inhibitor, type 1	236	1.53	0.34	1.27	1.28	2.90	1.44	0.52	0.84	0.79	0.75
p1K3	Pleckstrin	432	0.39	0.45	0.99	0.92	1.78	0.43	1.39	0.41	1.66	1.00
p1B3	Proline 4-hydroxylase, alpha polypeptide I	232	1.12	1.00	1.44	1.83	0.71	0.74	0.78	0.25	1.22	0.50
p1F20	Proline-rich protein with nuclear targeting signal	336	4.29	0.54	2.08	1.23	2.34	1.08	0.89	1.00	1.13	0.61
p1B22	Protease, serine, 11	356	3.86	0.86	9.76	2.40	1.17	0.95	1.18	0.88	0.79	1.00
p1C10	Regulator of G-protein signalling 1	376	0.26	0.12	1.09	1.10	1.93	0.14	0.42	0.12	0.44	1.00
p1P5	SCYA2	396	2.43	0.63	3.14	1.39	2.20	0.61	1.14	0.98	0.82	0.89
p1K8	SCYA4	408	1.00	0.56	2.48	2.29	1.94	0.54	1.94	0.68	1.75	1.62
p1I11	SECIS binding protein 2	60	1.49	0.90	1.00	0.98	1.09	0.36	2.31	2.24	2.10	1.49
p1I18	Selectin L	488	0.60	0.78	1.00	1.46	4.25	1.46	5.73	1.09	4.30	2.26
p1D3	Serine carboxypeptidase 1	96	0.76	0.09	0.91	0.98	0.76	0.50	0.98	1.00	1.13	0.95
p1J17	SLC6A1	438	0.11	0.39	0.85	1.42	0.80	3.56	3.08	1.17	4.08	1.00
p1F7	Spectrin, beta, non-erythrocytic 1	16	2.52	0.48	2.04	1.14	1.65	0.95	2.30	1.90	0.91	0.91
p1J18	Synaptopodin	440	0.07	0.31	0.99	1.44	0.68	3.03	3.51	1.40	4.54	1.29
p1J15	TERA protein	442	0.68	2.14	0.80	1.47	1.00	2.36	2.97	2.74	3.14	0.97
p1K4	TSC-22	444	2.92	0.46	1.40	2.19	0.85	1.05	0.56	1.12	0.35	0.88

TABLE 20: Genes up-regulated in response to TNFα

			Cytokine / % Oxygen			
			none	none	TNF	TNF
Clone	Gene Name	SeqID	20	0.1	20	0.1
p1C14	Abstrakt	384	1	7.89	4.63	7.87
p1D13	Adenylate kinase 3	78	1	0.85	2.36	2.47
p1A22	Adenylate kinase 3	264	1	1.53	2.69	3.72
p1B8	adipophilin	314	1	17.1	2.27	8.81
p1B7	adipophilin	314	1	13.4	2.17	5.86
p1A19	Aldolase C	260	1	6.61	2.57	6.31
p1N17	COX-2	238	1	1.04	0.91	2.24
p1C1	CXCR4	332	1	5.42	2.21	5.22
p1F4	CYP1	340	1	2.86	3.43	6.26
p1E3	CYP1B1	138	1	0.33	2.12	1.14
p1F16	CYP1B1	326	1	0.45	1.93	1.19
p2L23	endothelin 1	398	1	0.95	2.74	2.41
p1A14	Enolase 1	258	1	9.98	7.22	11.78
p1A11	GAPDH	254	1	7.87	3.60	5.90
p1C6	Glucose phosphate isomerase	368	1	5.18	2.58	3.61
p1D9	Hypothetical protein DKFZP564D116	28	1	2.37	2.48	3.16
p1F5	Hypothetical protein FLJ20281	12	1	3.84	2.05	3.78
p1B23	interleukin 1 receptor antagonist	358	1	3.46	3.43	5.35
p1B14	Interleukin 8	252	1	5.52	16.8	56.8
p1B16	Interleukin 8	252	1	2.55	9.64	23.3
p1B15	Interleukin 8	252	1	3.37	10.4	28.1
p1C13	Jk-recombination signal binding protein	382	1	5.82	4.77	8.75
p1A8	Lactate dehydrogenase A	224	1	24.8	4.08	15.1
p1A13	Phosphoglycerate kinase 1	256	1	7.29	2.73	4.65
p1B19	Plasminogen activator inhibitor, type 1	236	1	3.78	2.63	9.41
p1B18	Plasminogen activator inhibitor, type 1	236	1	4.92	2.23	6.55
p1C11	Polyubiquitin	378	1	2.80	2.06	3.03
p1B4	Proline 4-hydroxylase, alpha polypeptide II	350	1	6.15	3.09	5.80
p1F20	Proline-rich protein with nuclear targeting signal	336	1	4.69	2.18	6.45
p1I20	SCYA3L	470	1	0.77	3.97	3.61
p1K8	SCYA4	408	1	0.81	9.65	9.63
p1D3	Serine carboxypeptidase 1	96	1	3.74	2.37	3.55
p1A2	SLC2A3	248	1	16.0	2.68	15.5
p1F22	Sorting nexin 9	320	1	0.66	1.26	1.63
p1B10	Stearoyl-CoA desaturase	352	1	5.04	3.05	6.95

p1B17	Tissue factor	226	1	3.69	2.74	6.03
p1A20	Triosephosphate isomerase 1	262	1	16.1	8.30	16.2
p1E14	unknown mRNA (schizophrenia-linked)	98	1	3.30	3.03	3.26

TABLE 21: Genes down-regulated in response to TNFα

			Cytokine / % Oxygen			
			none	none	TNF	TNF
Clone	Gene Name	SeqID	20	0.1	20	0.1
p1E5	Hepcidin antimicrobial peptide	142	1	1.50	0.19	0.70
p1H2	Fatty acid binding protein 5	210	1	0.72	0.38	0.46
p1P5	SCYA2	396	1	0.29	0.45	0.26
p1J5	SCYA7	464	1	0.89	0.46	0.49

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TABLE 22: Genes up-regulated in response to IL-17

			Cytokine / % Oxygen			
			none	none	IL-17	IL-17
Clone	Gene Name	SeqID	20	0.1	20	0.1
p1J11	Fatty-acid-Coenzyme A ligase, long-chain 2	466	1	0.55	1.63	1.28
p1A11	GAPDH	254	1	0.78	2.60	1.84
p1C6	Glucose phosphate isomerase	368	1	0.84	2.14	1.43
p1I24	GRO1	486	1	1.02	2.29	1.28
p1I19	GRO2	482	1	1.02	2.26	1.43
p1B16	Interleukin 8	252	1	1.77	9.52	12.2
p1B15	Interleukin 8	252	1	1.54	7.36	9.71
p1B14	Interleukin 8	252	1	1.50	9.34	7.13
p1P5	SCYA2	396	1	0.24	2.12	0.58
p1K8	SCYA4	408	1	0.44	2.48	0.83

TABLE 23: Genes down-regulated in response to IL-17

			Cytokine / % Oxygen			
			none	none	IL-17	IL-17
Clone	Gene Name	SeqID	20	0.1	20	0.1
p1H8	ABL	182	1	1.08	0.08	0.09
p1J22	Neutral sphingomyelinase (N-SMase) activation associated factor	428	1	1.21	0.13	0.11
p1K14	Keratin 6B	422	1	1.27	0.15	0.15
p1J6	Hypothetical protein FLJ10206	40	1	1.28	0.22	0.23
p1I12	Hypothetical protein MGC4549	152	1	1.20	0.32	0.30
p1E15	cDNA YI27F12	108	1	1.58	0.21	0.56
p2A14	EST	446	1	1.10	0.56	0.40
p1G24	Glycogen synthase 1	276	1	1.45	0.34	0.65
p1C16	Decidual protein induced by progesterone	388	1	1.09	0.73	0.51
p1D8	Hypoxia-inducible protein 2	272	1	1.30	0.44	0.65
p1B18	Plasminogen activator inhibitor, type 1	236	1	1.10	0.49	0.78
p1H4	EST	214	1	1.13	0.49	0.58

TABLE 24: Genes up-regulated in response to IL-15

			Cytokine / % Oxygen			
			none	none	IL-15	IL-15
Clone	Gene Name	SeqID	20	0.1	20	0.1
p1A19	Aldolase C	260	1	0.50	0.35	1.30
p1J16	cDNA: FLJ23019 fis, clone LNG00916	454	1	0.80	5.76	7.27
p1D19	EST	144	1	1.00	2.27	1.39
p1J11	Fatty-acid-Coenzyme A ligase, long-chain 2	466	1	0.55	1.64	1.29
p1A11	GAPDH	254	1	0.78	0.52	4.32
p1C6	Glucose phosphate isomerase	368	1	0.84	0.57	3.13
p1H5	Hypothetical protein FLJ22690	206	1	0.94	2.37	1.59
p1A23	Metallothionein 2A	266	1	1.41	1.26	3.08
p1P5	SCYA2	396	1	0.24	4.51	1.37
p1J5	SCYA7	464	1	0.66	3.27	1.61
p1I21	SCYA8	480	1	0.37	3.77	1.55
p1I7	Uridine 5' monophosphate hydrolase 1	50	1	0.84	4.98	3.61

TABLE 25: Genes down-regulated in response to IL-15

			Cytokine / % Oxygen			
			none	none	IL-15	IL-15
Clone	Gene Name	SeqID	20	0.1	20	0.1
p1H8	ABL	182	1	1.08	1.22	0.09
p1C14	Abstrakt	384	1	0.69	0.40	1.24
p1B8	Adipophilin	314	1	1.08	0.23	1.42
p1B7	Adipophilin	314	1	1.10	0.30	2.02
p1B6	Adipophilin	314	1	0.98	0.37	2.39
p1B9	Adipophilin	314	1	1.46	0.41	1.66
p1A19	Aldolase C	260	1	0.50	0.35	1.30
p1C7	D123	370	1	0.53	0.47	0.90
p1H6	EST	192	1	1.46	1.95	0.67
p2A14	EST	446	1	1.10	1.08	0.51
p1G24	Glycogen synthase 1	276	1	1.45	0.88	0.55
p1J6	Hypothetical protein FLJ10206	40	1	1.28	1.28	0.18
p1I12	Hypothetical protein MGC4549	152	1	1.20	1.64	0.28
p1D8	Hypoxia-inducible protein 2	272	1	1.30	1.25	0.62
p1K14	Keratin 6B	422	1	1.27	1.48	0.11
p1A8	Lactate dehydrogenase A	224	1	1.31	0.48	2.29
p1A9	Lactate dehydrogenase A	224	1	1.95	0.49	1.83
p1J22	Neutral sphingomyelinase (N-SMase) activation associated factor	428	1	1.21	1.51	0.11
p1B4	Proline 4-hydroxylase, alpha polypeptide II	350	1	0.81	0.50	1.34
p1A20	Triosephosphate isomerase 1	262	1	0.81	0.36	1.22

TABLE 26 cross-references all protein and nucleotide sequences (SEQ ID Nos.) that are referenced herein to accession numbers in public databases available as of 8.12.00.

	Hypoxia	PROTEIN		NUCLEOTIDE	
TITLE	response	SEQ ID	ACCESSION	SEQ ID	ACCESSION
cDNA FLJ13611 fis, clone PLACE1010802	Increase	1	BAB14633	2	AK023673
Hypothetical protein FLJ20037	Increase	3	BAA90903	4	AK000044
hypothetical protein DKFZp434P0116	Increase	5	CAB70863	6	AL137661
KIAA0212	Increase	7	BAA13203	8	D86967
KIAA0914	Increase	9	BAA74937	10	AB020721
Hypothetical protein FLJ20281	Increase	11	NP_060212	12	NM_017742
KIAA0876	Increase	13	BAA74899	14	AB020683
cDNA FLJ13700 fis, clone PLACE2000216	Increase	15	(nearest=Q01082)	16	AK023762
DKFZP586G1122 protein	Increase	17	CAB55938	18	AL117462
Putative zinc finger protein LOC55818	Increase	19	AAF67005	20	AF155648
hypothetical protein PRO0823	Increase	21	AAF71073	22	AF116653
Hypothetical protein FLJ10134.	Increase	23	BAA91458	24	AK000996
Hypothetical protein FLJ20500	Increase	25	BAA91214	26	AK000507
DKFZP564D116 protein	Increase	27	CAB43242	28	AL050022
KIAA1376 protein	Increase	29	BAA92614	30	AB037797
Hypothetical protein KIAA0127	Increase	31	BAA09476	32	D50917
Hypothetical protein FLJ20308	Increase	33	BAA91078	34	AL137263
Hypothetical nuclear factor SBBI22	Repression	35	NP_065128	36	NM_020395
DKFZP434I116 protein	Repression	37	CAB55922	38	AL117434
Hypothetical prot. FLJ10206	Repression	39	NP_060495	40	NM_018025
hypothetical protein FLJ10815	Repression	41	BAA91830	42	AK001677
Hypothetical protein FLJ11100	Repression	43	BAA92003	44	AK001962

hypothetical protein FLJ2064	Repression	45	NP_060387	46	NM_017917
Hypothetical protein HSPC111	Repression	47	NP_057475	48	NM_016391
hypothetical protein LOC51251	Repression	49	NP_057573	50	NM_016489
KIAA0014	Repression	51	BAA04946	52	D25216
Hypothetical protein HSPC196	Repression	53	NP_057548	54	NM_016464
Hypothetical protein FLJ11296	Repression	55	BAA92115	56	AK002158
Hypothetical protein bA395L14	Repression	57	CAB62980	58	AL022311
cDNA FLJ13016 fis, clone NT2RP3000624	Repression	59	BAB14393	60	AK023078
cDNA DKFZp586H0324 clone DKFZp586H0324	Increase	61	none	62	AL110163
Clone 23785	Increase	63	none	64	AF035307
cDNA DKFZp586E1624	Increase	65	none	66	AL110152
cDNA FLJ14162 fis, clone NT2RM4002504	Increase	67	none	68	AK024224
cDNA DKFZp434E1723 (clone DKFZp434E1723)	Increase	69	none	70	AL137473
cDNA FLJ11041 fis, clone PLACE1004405	Increase	71	none	72	AK001903
cDNA FLJ10433 fis NT2RP1000478	Increase	73	none	74	AK001295
cDNA DKFZp434O071	Increase	75	none	76	AF125392
cDNA FLJ23313 fis, clone HEP11919	Increase	77	none	78	AK026966
ESTs	Increase	79	none	80	R62339
ESTs	Increase	81	none	82	AA489477
ESTs	Increase	83	none	84	R06601
ESTs	Increase	85	none	86	R00332
ESTs	Increase	87	none	88	AA463469
ESTs	Increase	89	none	90	H56028
ESTs	Increase	91	none	92	AA293300
ESTs	Increase	93	none	94	AW250104
ESTs	Increase	95	none	96	BE382614
ESTs	Increase	97	none	98	H59618
ESTs	Increase	99	none	100	AA449703
ESTs	Increase	101	none	102	AA521311
ESTs	Increase	103	none	104	W69170

ESTs	Increase	105	none	106	R51835
ESTs	Increase	107	none	108	H87770
ESTs	Increase	109	none	110	R69248
ESTs	Increase	111	none	112	T68844
ESTs	Increase	113	none	114	AA454177
ESTs	Increase	115	none	116	AA026562
ESTs	Increase	117	none	118	T73780
ESTs	Increase	119	none	120	AA401496
ESTs	Increase	121	none	122	AA489636
ESTs	Increase	123	none	124	AA446361
ESTs	Increase	125	none	126	AA931411
ESTs	Increase	127	none	128	R24223
ESTs	Increase	129	none	130	R22252
ESTs	Increase	131	none	132	AA612751
ESTs	Increase	133	none	134	AW964331
ESTs	Increase	135	none	136	AI018611
ESTs	Increase	137	none	138	AA451886
ESTs	Increase	139	none	140	R06520
ESTs	Increase	141	none	142	T48278
ESTs	Increase	143	none	144	R68736
cDNA FLJ14028 fis, clone HEMBA1003838	Repression	145	none	146	AK024090
cDNA DKFZp564D016 (clone DKFZp564D016)	Repression	147	none	148	AL050021
cDNA FLJ11302 fis, clone PLACE1009971	Repression	149	none	150	AK002164
NEDO FLJ10309 fis cl NT2RM2000287	Repression	151	none	152	AK001171
Sequence from clone RP11-394O2 on ch 20	Repression	153	none	154	AK022731
ESTs	Repression	155	none	156	AA420992
ESTs	Repression	157	none	158	AA693797
ESTs	Repression	159	none	160	AA456437
ESTs	Repression	161	none	162	AA429367
ESTs	Repression	163	none	164	AA434382

ESTs	Repression	165	none	166	AA664228
ESTs	Repression	167	none	168	R44397
ESTs	Repression	169	none	170	AA923509
ESTs	Repression	171	none	172	W87747
ESTs	Repression	173	none	174	AA973568
ESTs	Repression	175	none	176	T98529
ESTs	Repression	177	none	178	AA022679
ESTs	Repression	179	none	180	H17921
ESTs	Repression	181	none	182	R00766
ESTs	Repression	183	none	184	W91958
ESTs	Repression	185	none	186	R63694
ESTs	Repression	187	none	188	AA425386
ESTs	Repression	189	none	190	AA909912
ESTs	Repression	191	none	192	T99032
ESTs	Repression	193	none	194	H52503
ESTs	Repression	195	none	196	AA127017
ESTs	Repression	197	none	198	R38647
ESTs	Repression	199	none	200	T87233
ESTs	Repression	201	none	202	AA130351
ESTs	Repression	203	none	204	H49601
ESTs	Repression	205	none	206	AA598952
ESTs	Repression	207	none	208	AA991868
ESTs	Repression	209	none	210	T60111
ESTs	Repression	211	none	212	AA897090
ESTs	Repression	213	none	214	AA679939
ESTs	Repression	215	none	216	AA630167
BCL2/adenovirus E1B 19kD-interacting protein 3-like	Increase	217	NP_004322	218	NM_004331
Solute carrier family 2, member 1	Increase	219	NP_006507	220	NM_006516
PDGF beta	Increase	221	NP_002599	222	NM_002608
lactate dehydrogenase A	Increase	223	NP_005557	224	NM_005566

Tissue factor	Increase	225	NP_001984	226	NM_001993
Vascular endothelial growth factor	Increase	227	NP_003367	228	NM_003376
RTP / NDRG1	Increase	229	NP_006087	230	NM_006096
Procollagen-proline 4-hydroxylase alpha 1	Increase	231	NP_000908	232	NM_000917
BCL2/adenovirus E1B-interacting protein 3	Increase	233	NP_004043	234	NM_004052
Plasminogen activator inhibitor, type I	Increase	235	AAA60003	236	M16006
Cyclooxygenase 2	Increase	237	AAA57317	238	U04636
Metallothionein 1H	Increase	239	CAA46046	240	X64834
Metallothionein 1L	Increase	241	P80297	242	AJ011772
Metallothionein-IG	Increase	243	AAA59873	244	J03910
Metallothionein 1E (functional)	Increase	245	AAA59587	246	M10942
Solute carrier family 2, member 3	Increase	247	AAB61083	248	M20681
Hexokinase 2	Increase	249	CAA86511	250	Z46376
Interleukin 8	Increase	251	CAA68742	252	Y00787
Glyceraldehyde-3-phosphate dehydrogenase	Increase	253	NP_002037	254	NM_002046
Phosphoglycerate kinase 1	Increase	255	NP_000282	256	NM_000291
Enolase 1	Increase	257	NP_001419	258	NM_001428
aldolase C, fructose-bisphosphate (ALDOC)	Increase	259	NP_005156	260	NM_005165
Triosephosphate isomerase 1 (TPI1)	Increase	261	NP_000356	262	NM_000365
Adenylate kinase 3 (AK3)	Increase	263	NP_037542	264	NM_013410
Metallothionein-2a	Increase	265	AAA59583	266	J00271
Osteopontin	Increase	267	CAA31984	268	X13694
Granulin	Increase	269	AAA58617	270	AK000607
Hypoxia-inducible protein 2	Increase	271	NP_037464	272	NM_013332
Enolase 2, (gamma, neuronal)	Increase	273	NP_001966	274	NM_001975
Glycogen synthase 1 (muscle)	Increase	275	AAB60385	276	U32573
Activated leucocyte cell adhesion molecule	Increase	277	NP_001618	278	NM_001627
MAX-interacting protein 1	Increase	279	NP_005953	280	NM_005962
Nuclear receptor co-repressor	Increase	281	NP_006302	282	NM_006311
Chitinase 3-like 2	Increase	283	AAC50597	284	U49835
BACH1 transcription factor	Increase	285	NP_001177	286	NM_001186

Phosphoglucomutase 1	Increase	287	NP_002624	288	NM_002633
CGI-109 protein	Increase	289	AAD34104	290	AF151867
SAP30	Increase	291	NP_003855	292	NM_003864
ATP-binding cassette transporter-1	Increase	293	NP_005493	294	NM_005502
SEC24 protein	Increase	295	CAA10334	296	AJ131244
Trinucleotide repeat containing 3	Increase	297	NP_005869	298	NM_005878
Post-synaptic density protein 95	Increase	299	AAC52113	300	U83192
Tumor protein D52	Increase	301	NP_005070	302	NM_005079
Cyclin-dependent kinase inhibitor p27kip1	Increase	303	NP_004055	304	NM_004064
phosphoinositide-3-kinase, catalytic, beta	Increase	305	NP_006210	306	NM_006219
Solute carrier family 5, member 3	Increase	307	NP_008864	308	NM_006933
PSCDBP	Increase	309	NP_004279	310	NM_004288
Solute carrier family 2, member 5	Increase	311	AAA52570	312	M55531
Adipophilin	Increase	313	NP_001113	314	NM_001122
Early development regulator 2	Increase	315	NP_004418	316	NM_004427
B-cell translocation gene 1,	Increase	317	NP_001722	318	NM_001731
SH3PX1	Increase	319	NP_057308	320	NM_016224
Cyclin G2	Increase	321	NP_004345	322	NM_004354
NAG-5 protein	Increase	323	NP_057530	324	NM_016446
Cytochrome P450 IB1 (dioxin-inducible)	Increase	325	NP_000095	326	NM_000104
Butyrate response factor 1	Increase	327	NP_004917	328	NM_004926
p8 protein (candidate of metastasis 1)	Increase	329	NP_036517	330	NM_012385
chemokine (C-X-C motif), receptor 4 (CXCR4)	Increase	331	NP_003458	332	NM_003467
solute carrier family 16, member 6	Increase	333	AAC52014	334	U79745
Proline-rich protein with nuclear targeting signal (B4-2)	Increase	335	NP_006804	336	NM_006813
RNA helicase-related protein	Increase	337	AAC32396	338	AF083255
Cytochrome P450, subfamily XXVIIB, polypeptide 1	Increase	339	BAA22656	340	AB005989
SHB adaptor protein	Increase	341	CAA53091	342	X75342
Papillomavirus regulatory factor (PRF-1)	Increase	343	NP_061130	344	NM_018660
SLC31A2/ hCTR1	Increase	345	NP_001851	346	NM_001860
UDP-glucose pyrophosphorylase 2 (UGP2)	Increase	347	NP_006750	348	NM_006759

Proline 4-hydroxylase, alpha polypeptide II	Increase	349	NP_004190	350	NM_004199
Stearoyl-CoA desaturase	Increase	351	BAA93510	352	AB032261
Diacylglycerol kinase, zeta	Increase	353	NP_003637	354	NM_003646
Serine protease 11	Increase	355	BAA13322	356	Y07921
IL-1 receptor antagonist, alternatively spliced forms	Increase	357	AAB92268, AAB92269, AAB92270	358	U65590
NS1-binding protein	Increase	359	NP_006460	360	NM_006469
Activin A receptor type I	Increase	361	NP_001096	362	NM_001105
FGF receptor activating protein 1 (FRAG1)	Increase	363	AAF19156	364	AF159621
Galectin-8	Increase	365	AAF19370	366	AF193806
Glucose 6-phosphate isomerase	Increase	367	NP_000166	368	NM_000175
D123 protein	Increase	369	AAC34738	370	U27112
Dec1.	Increase	371	NP_003661	372	NM_003670
Rab-8b	Increase	373	NP_057614	374	NM_016530
BL34	Increase	375	AAB26289	376	S59049
Polyubiquitin UbC	Increase	377	BAA23632	378	AB009010
Integrin alpha 5	Increase	379	NP_002196	380	NM_002205
Jk-recombination signal binding protein	Increase	381	AAA60258	382	L07872
DEAD-box protein abstrakt	Increase	383	NP_057306	384	NM_016222
High mobility group 2 protein	Increase	385	AAA58659	386	M83665
Decidual protein induced by progesterone	Increase	387	NP_008952	388	NM_007021
GM2 ganglioside activator protein.	Increase	389	CAA43993, CAA43994	390	X62078
CCR4 associated factor 1 (CAF1)	Increase	391	AAD02685	392	AF053318
Nucleoside phosphorylase	Repression	393	NP_000261	394	NM_000270
Monocyte chemotactic protein 1	Repression	395	NP_002973	396	NM_002982
Endothelin 1	Repression	397	NP_001946	398	NM_001955
Heat shock 70kD protein 4	Repression	399	AAA02807	400	L12723
Annexin A1	Repression	401	NP_000691	402	NM_000700

p67 myc protein	Repression	403	CAA25105, CAA25106	404	X00364
Alpha-2-macroglobulin	Repression	405	NP_000005	406	NM_000014
Macrophage inflammatory protein 1b	Repression	407	NP_002975	408	NM_002984
Sex hormone-binding globulin	Repression	409	NP_001031	410	NM_001040
ATP-binding cassette, sub-family E (OABP), member 1	Repression	411	NP_002931	412	NM_002940
Chaperonin / Tcp zeta 1	Repression	413	NP_001753	414	NM_001762
Colony stimulating factor 1 (macrophage)	Repression	415	AAA59573	416	M27087
Dendritic cell protein (GA17)	Repression	417	NP_006351	418	NM_006360
G protein-coupled receptor 44	Repression	419	NP_004769	420	NM_004778
Keratin 6A	Repression	421	NP_005545	422	NM_005554
lymphocyte adaptor protein	Repression	423	NP_005466	424	NM_005475
Neuro-oncological ventral antigen 1	Repression	425	AAA16022	426	U04840
N-SMase / FAN	Repression	427	CAA65405	428	X96586
Peptidylprolyl isomerase F (cyclophilin F)	Repression	429	NP_005720	430	NM_005729
PLECKSTRIN	Repression	431	NP_002655	432	NM_002664
High affinity immunoglobulin epsilon receptor beta	Repression	433	AAF17243	434	AF201951
Ribosomal protein L44	Repression	435	NP_000992	436	NM_001001
Solute carrier family 6 No1	Repression	437	NP_003033	438	NM_003042
Synaptopodin	Repression	439	NP_009217	440	NM_007286
TERA protein	Repression	441	AAF87322	442	AF212220
TGF beta-stimulated protein TSC-22	Repression	443	NP_006013	444	NM_006022
Tubulin, beta, 2	Repression	445	NP_006079	446	NM_006088
Calgranulin A	Repression	447	NP_002955	448	NM_002964
Replication factor C (145 KDa)	Repression	449	NP_002904	450	NM_002913
Signal recognition particle 19 kD protein	Repression	451	NP_003126	452	NM_003135
Transcription factor SUPT3H	Repression	453	NP_003590	454	NM_003599
Proteasome component C9	Repression	455	NP_002780	456	NM_002789
Maf-related leucine zipper homolog	Repression	457	NP_005452	458	NM_005461
dynein, cytoplasmic, light intermediate polypeptide 2	Repression	459	NP_006132	460	NM_006141
Heterochromatin-like protein 1	Repression	461	NP_057671	462	NM_016587

Monocyte chemotactic protein 3	Repression	463	NP_006264	464	NM_006273
Fatty-acid-Coenzyme A ligase, long-chain 2	Repression	465	BAA00931	466	D10040
Programmed cell death 5 / TFAR19	Repression	467	NP_004699	468	NM_004708
Small inducible cytokine A3	Repression	469	AAA36316	470	M23452
Cytochrome c oxidase subunit VIc	Repression	471	NP_004365	472	NM_004374
NASP histone-binding prot.	Repression	473	NP_002473	474	NM_002482
Ecotropic viral integration site 2A	Repression	475	NP_055025	476	NM_014210
Sjogren syndrome antigen B	Repression	477	AAA51885	478	J04205
Monocyte chemotactic protein 2	Repression	479	NP_005614	480	NM_005623
GRO2/ macrophage inflammatory protein 2a	Repression	481	NP_002080	482	NM_002089
Small nuclear ribonucleoprotein SM D 1	Repression	483	NP_008869	484	NM_006938
GRO1/ macrophage inflammatory protein 2 precursor	Repression	485	NP_001502	486	NM_001511
Lymphocyte adhesion molecule 1	Repression	487	NP_000646	488	NM_000655

TABLE 27 cross-references all protein and nucleotide sequences (SEQ ID Nos.) that are referenced herein to accession numbers in public databases available as of 8.12.01.

Clone ID	New Name	Old Name	Protein Seq ID	Protein Accession	Nucleotide Seq ID	GenBank Locus
p1F12	Hypothetical protein FLJ13611	cDNA FLJ13611 fis, clone PLACE1010802	1	NP_079217	2	NM_024941
p1F2	Hypothetical protein FLJ20037	Hypothetical protein FLJ20037	3	CAB65981	4	NM_017633
p1F10	Hypothetical protein DKFZp434P0116	hypothetical protein DKFZp434P0116	5	T46364	6	NM_017593
p1F19	Hypothetical protein KIAA0212	KIAA0212	7	BAA13203	8	NM_014674
p1F8	Hypothetical protein KIAA0914	KIAA0914	9	NP_055698	10	NM_014883
p1F5	Hypothetical protein FLJ20281	Hypothetical protein FLJ20281	11	XP_008736	12	NM_017742
p1F18	Hypothetical protein KIAA0876	KIAA0876	13	BAA74899	14	XM_035625
p1F7	Spectrin, beta, non-erythrocytic 1	cDNA FLJ13700 fis, clone PLACE2000216	15	NP_003119	16	NM_003128
p1F21	Hematopoietic Zinc finger protein	DKFZP586G1122 protein	17	AAL08625	18	AK024404
p1F9	Hypothetical protein KIAA0742	Putative zinc finger protein LOC55818	19	NP_060903	20	AB018285
p1E13	Hypothetical protein PRO0823	hypothetical protein PRO0823	21	AAF71073	22	AF116653
p1D1	Hypothetical protein FLJ10134	Hypothetical protein FLJ10134	23	NP_060474	24	NM_018004
p1D2	Hypothetical protein FLJ10134	Hypothetical protein FLJ10134	23	NP_060474	24	NM_018004
p1D4	Hypothetical protein FLJ20500	Hypothetical protein FLJ20500	25	NP_061931	26	NM_019058
p1D9	Hypothetical protein DKFZP564D116	DKFZP564D116 protein	27	T08708	28	AL050022
p1D12	Hypothetical protein KIAA1376	KIAA1376 protein	29	BAA92614	30	AB037797
p1D15	TRIP-Br2	Hypothetical protein KIAA0127	31	NP_055570	32	NM_014755
p1D16	Hypothetical protein FLJ20308	Hypothetical protein FLJ20308	33	XP_039852	34	AK000315
p1J13	Hypothetical nuclear factor SBBI22	Hypothetical nuclear factor SBBI22	35	NP_065128	36	NM_020395
p1I22	Hypothetical protein KIAA1429	DKFZP434I116 protein	37	BAA92667	38	AB037850
p1J6	Hypothetical protein FLJ10206	Hypothetical prot. FLJ10206	39	AAH06108	40	NM_018025

p1I5	Hypothetical protein FLJ10815	hypothetical protein FLJ10815	41	BAA91830	42	NM_018231
p1I13	Hypothetical protein FLJ11100	Hypothetical protein FLJ11100	43	NP_060701	44	NM_018321
p1I17	Hypothetical protein FLJ20644	hypothetical protein FLJ2064	45	NP_060387	46	NM_017917
p1I15	Hypothetical protein CGI-117	Hypothetical protein HSPC111	47	Q9Y3C1	48	NM_016391
p1I7	Uridine 5' monophosphate hydrolase 1	hypothetical protein LOC51251	49	NP_057573	50	NM_016489
	Hypothetical protein KIAA0014	KIAA0014	51	NP_055480	52	NM_014665
p1I4	Hypothetical protein HSPC196	Hypothetical protein HSPC196	53	NP_057548	54	NM_016464
p1I8	Hypothetical protein FLJ11296	Hypothetical protein FLJ11296	55	XP_004747	56	NM_018384
p1I16	Hypothetical protein KIAA1668	Hypothetical protein bA395L14	57	BAB33338	58	AB051455
p1I11	SECIS binding protein 2	cDNA FLJ13016 fis, clone NT2RP3000624	59	AAK57518	60	AF380995
p1E8	cDNA: FLJ22249 fis, clone HRC02674	cDNA DKFZp586H0324 clone DKFZp586H0324	61	None	62	AK025902
p1E18	Plexin C1	Clone 23785	63	NP_005752	64	NM_005761
p1E16	cDNA DKFZp586E1624	cDNA DKFZp586E1624	65	None	66	AL110152
p1D5	ERO1 (S. cerevisiae)-like	cDNA FLJ14162 fis, clone NT2RM4002504	67	NP_055399	68	NM_014584
p1D6	ERO1 (S. cerevisiae)-like	cDNA FLJ14162 fis, clone NT2RM4002504	67	NP_055399	68	NM_014584
p1E12	Hypothetical protein DKFZP434E1723	cDNA DKFZp434E1723 (clone DKFZp434E1723)	69	XP_05338	70	BC010005
p1E10	cDNA FLJ11041 fis, clone PLACE1004405	cDNA FLJ11041 fis, clone PLACE1004405	71	None	72	AK001903
p1C21	Tubulin, beta, 4	cDNA FLJ10433 fis NT2RP1000478	73	NP_006077	74	NM_006086
p1D10	Insulin induced protein 2	cDNA DKFZp434O071	75	AAD43048	76	AF125392
p1D13	Adenylate kinase 3	cDNA FLJ23313 fis, clone HEP11919	77	NP_037542	78	NM_013410
p1E9	Novel PI-3-kinase adapter	ESTs	79	None	80	R62339
p1F1	EST	ESTs	81	None	82	AA489477
p1E7	Novel Metallothionein	ESTs	83	None	84	R06601

p1E6	EGL nine (C.elegans) homolog 3	ESTs	85	NP_071356	86	NM_022073
p2B1	PRAME	ESTs	87	NP_006106	88	NM_006115
p1D14	C1orf12	ESTs	89	NP_071334	90	NM_022051
p1D17	Semaphorin 4b	ESTs	91	BAB21836	92	AB051532
p1P14	Semaphorin 4b	ESTs	91	BAB21836	92	AB051532
p1C24	SLC25A19	ESTs	93	NP_068380	94	NM_021734
p1D3	Serine carboxypeptidase 1	ESTs	95	NP_067639	96	NM_021626
p1E14	Unknown mRNA (schizophrenia-linked)	ESTs	97	None	98	AY010112
p1E20	Myo-inositol monophosphatase A3	ESTs	99	AAK52336	100	NM_017813
p2A24	EST	ESTs	101	None	102	AA521314
p1E17	Hypothetical protein FLJ31668	ESTs	103	BAB71124	104	AK056230
p1E19	EST	ESTs	105	None	106	R51835
p1E15	cDNA YI27F12	ESTs	107	None	108	AF075018
p1E11	EST	ESTs	109	None	110	R69248
p1E23	cDNA FLJ14041 fis, clone HEMBA1005780	ESTs	111	None	112	AK024103
p1E21	Glutamate-cysteine ligase, modifier subunit	ESTs	113	NP_002052	114	NM_002061
p1D23	PTEN	ESTs	115	NP_000305	116	NM_000314
p1D24	EST	ESTs	117	None	118	T73780
p1D22	MAX-interacting protein 1	ESTs	119	NP_005953	120	NM_005962
p1E2	Mannosidase, alpha, class 1A, member 1	ESTs	121	NP_005898	122	NM_005907
p1E1	EST	ESTs	123	None	124	AA446361
p1E4	EST	ESTs	125	None	126	AA931411
p1D18	cDNA FLJ13443 fis, clone PLACE1002853	ESTs	127	None	128	AK023505
p1D21	Hypothetical protein FLJ22622	ESTs	129	BAB15424	130	NM_025151
p1C22	CD84-H1	ESTs	131	AAK69052	132	AF275725
p1C23	Hypothetical protien FLJ12832	ESTs	133	XP_043394	134	AK022894
p1D11	EST	ESTs	135	None	136	AA251748

p1E3	CYP1B1	ESTs	137	NP_000095	138	NM_000104
p1D20	Hypothetical protein KIAA1125	ESTs	139	XP_012932	140	AB032951
p1E5	Hepcidin antimicrobial peptide	ESTs	141	NP_066998	142	NM_021175
p1D19	EST	ESTs	143	None	144	R68736
p2A15	Sialyltransferase	cDNA FLJ14028 fis, clone HEMBA1003838	145	NP_006447	146	NM_006456
p1I14	cDNA DKFZp564D016	cDNA DKFZp564D016 (clone DKFZp564D016)	147	None	148	AL050021
p1I2	cDNA FLJ11302 fis, clone PLACE1009971	cDNA FLJ11302 fis, clone PLACE1009971	149	None	150	AK002164
p1I12	Hypothetical protein MGC4549	NEDO FLJ10309 fis cl NT2RM2000287	151	XP_032794	152	NM_032377
p1I3	ELMO2	Sequence from clone RP11-394O2 on ch 20	153	AAL14467	154	XM_012933
p1I10	EST	ESTs	155	None	156	AA420992
p1H18	Ubiquitin specific protease 7	ESTs	157	NP_003461	158	NM_003470
p1H24	Nucleolar phosphoprotein Nopp34	ESTs	159	NP_115766	160	NM_032390
p1E22	cDNA FLJ13618 fis, clone PLACE1010925	ESTs	161	None	162	AK023680
p1H21	Hypothetical protein FLJ13511	ESTs	163	NP_149014	164	NM_033025
p1I1	Ribosomal RNA intergenic spacer	ESTs	165	None	166	AA664228
p1H14	EST	ESTs	167	None	168	R44397
p1H11	Carboxypeptidase M	ESTs	169	NP_001865	170	NM_001874
p1H17	EST	ESTs	171	None	172	W87747
p1H12	EST	ESTs	173	None	174	AA973568
p1H7	EST	ESTs	175	None	176	T98529
p1H15	EST	ESTs	177	None	178	AA022679
p1H20	EST	ESTs	179	None	180	H17921
p1H8	ABL	ESTs	181	NP_009297	182	NM_007313
p1H16	EST	ESTs	183	None	184	W91958
p1H9	EST	ESTs	185	None	186	R63694

p1H23	Hypothetical protein FLJ21094	ESTs	187	AAH14003	188	AK024747
p1H10	EST	ESTs	189	None	190	AA909912
p1H6	EST	ESTs	191	None	192	T99032
p1H13	EST	ESTs	193	None	194	H52503
p1H19	EST	ESTs	195	None	196	AA127017
p1G22	EST	ESTs	197	None	198	R38647
p1G21	EST	ESTs	199	None	200	T87233
p1H1	Hypothetical protein FLJ10826	ESTs	201	BAB14226	202	NM_018233
p1G20	cDNA YO23H03	ESTs	203	None	204	AF075053
p1H5	Hypothetical protein FLJ22690	ESTs	205	NP_078987	206	NM_024711
p1G19	Mitochondrion sequence	ESTs	207	AAH05845	208	BC005845
p1H2	Fatty acid binding protein 5	ESTs	209	NP_001435	210	NM_001444
p1G18	Mitochondrion sequence	ESTs	211	None	212	BC001612
p1H4	EST	ESTs	213	None	214	AA679939
p1H3	EST	ESTs	215	None	216	AA630167
	BCL2/adenovirus E1B 19kD-interacting protein 3-like	BCL2/adenovirus E1B 19kD-interacting protein 3-like	217	NP_004322	218	NM_004331
	SLC2A1	Solute carrier family 2, member 1	219	NP_006507	220	NM_006516
p1P3	PDGFB	PDGF beta	221	NP_148937	222	NM_033016
p1A8	Lactate dehydrogenase A	lactate dehydrogenase A	223	NP_005557	224	NM_005566
p1A9	Lactate dehydrogenase A	lactate dehydrogenase A	223	NP_005557	224	NM_005566
p1B17	Tissue factor	Tissue factor	225	NP_001984	226	NM_001993
p1O20	VEGF	Vascular endothelial growth factor	227	NP_003367	228	NM_003376
p1B2	N-myc downstream regulated	RTP/NDRG1	229	NP_006087	230	NM_006096
p1B3	Proline 4-hydroxylase, alpha polypeptide I	Procollagen-proline 4-hydroxylase alpha 1	231	NP_000908	232	NM_000917
	BCL2/adenovirus E1B-interacting protein 3	BCL2/adenovirus E1B-interacting protein 3	233	NP_004043	234	NM_004052
p1B18	Plasminogen activator inhibitor, type 1	Plasminogen activator inhibitor, type I	235	NP_000593	236	NM_000602
p1B19	Plasminogen activator inhibitor, type	Plasminogen activator inhibitor,	235	NP_000593	236	NM_000602

	1	type I				
p1N17	COX-2	Cyclooxygenase 2	237	NP_000954	238	NM_000963
p1A24	Metallothionein 1H	Metallothionein 1H	239	NP_005942	240	NM_005951
	Metallothionein 1L	Metallothionein 1L	241	NP_002441	242	NM_002450
p1B1	Metallothionein 1G	Metallothionein-IG	243	NP_005941	244	NM_005950
	Metallothionein 1E (functional)	Metallothionein 1E (functional)	245	None	246	AA872383
p1A1	SLC2A3	Solute carrier family 2, member 3	247	NP_008862	248	NM_006931
p1A2	SLC2A3	Solute carrier family 2, member 3	247	NP_008862	248	NM_006931
p1A3	SLC2A3	Solute carrier family 2, member 3	247	NP_008862	248	NM_006931
p1A4	SLC2A3	Solute carrier family 2, member 3	247	NP_008862	248	NM_006931
p1A15	Hexokinase-2	Hexokinase 2	249	NP_000180	250	NM_000189
p1A16	Hexokinase-2	Hexokinase 2	249	NP_000180	250	NM_000189
p1A17	Hexokinase-2	Hexokinase 2	249	NP_000180	250	NM_000189
p1A18	Hexokinase-2	Hexokinase 2	249	NP_000180	250	NM_000189
p1B14	Interleukin 8	Interleukin 8	251	NP_000575	252	NM_000584
p1B15	Interleukin 8	Interleukin 8	251	NP_000575	252	NM_000584
p1B16	Interleukin 8	Interleukin 8	251	NP_000575	252	NM_000584
p1A11	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	253	NP_002037	254	NM_002046
p1A12	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	253	NP_002037	254	NM_002046
p1A13	Phosphoglycerate kinase 1	Phosphoglycerate kinase 1	255	NP_000282	256	NM_000291
p1A14	Enolase 1	Enolase 1	257	NP_001419	258	NM_001428
p1A19	Aldolase C	aldolase C, fructose-bisphosphate (ALDOC)	259	NP_005156	260	NM_005165
p1A20	Triosephosphate isomerase 1	Triosephosphate isomerase 1 (TPI1)	261	NP_000356	262	NM_000365
p1A22	Adenylate kinase 3	Adenylate kinase 3 (AK3)	263	NP_037542	264	NM_013410
p1A23	Metallothionein 2A	Metallothionein-2a	265	NP_005944	266	NM_005953
p1B20	Osteopontin	Osteopontin	267	NP_000573	268	NM_000582
p1B21	Osteopontin	Osteopontin	267	NP_000573	268	NM_000582
p1C17	Granulin	Granulin	269	NP_002078	270	NM_002087
p1C18	Granulin	Granulin	269	NP_002078	270	NM_002087

p1D8	Hypoxia-inducible protein 2	Hypoxia-inducible protein 2	271	NP_037464	272	NM_013332
p1A10	Enolase 2	Enolase 2, (gamma, neuronal)	273	NP_001966	274	NM_001975
p1G24	Glycogen synthase 1	Glycogen synthase 1 (muscle)	275	NP_002094	276	NM_002103
p1G23	ALCAM	Activated leucocyte cell adhesion molecule	277	NP_001618	278	NM_001627
p1G5	MAX-interacting protein 1	MAX-interacting protein 1	279	NP_005953	280	NM_005962
p1G7	EST	Nuclear receptor co-repressor	281	None	282	BC008022
p2A23	Chitinase 3-like 2	Chitinase 3-like 2	283	NP_003991	284	NM_004000
p1G1	BACH1	BACH1 transcription factor	285	NP_001177	286	NM_001186
p1G15	Phosphoglucomutase 1	Phosphoglucomutase 1	287	NP_002624	288	NM_002633
p1F23	Hypothetical protein LOC51014	CGI-109 protein	289	Q9Y3B3	290	AF151867
p1G8	Sin3-associated polypeptide	SAP30	291	NP_003855	292	NM_003864
p1G13	ABCA1	ATP-binding cassette transporter-1	293	NP_005493	294	NM_005502
p1G10	SEC24 member A	SEC24 protein	295	CAA10334	296	AJ131244
p1F24	Glia-derived nexin	Trinucleotide repeat containing 3	297	AAA35883	298	M17783
p1G2	Postsynaptic density-95	Post-synaptic density protein 95	299	NP_001356	300	NM_001365
p1G11	Tumor protein D52	Tumor protein D52	301	NP_005070	302	NM_005079
p1G16	p27, Kip1	Cyclin-dependent kinase inhibitor p27kip1	303	NP_004055	304	NM_004064
p1G9	PI-3-kinase, catalytic, beta polypeptide	phosphoinositide-3-kinase, catalytic, beta	305	NP_006210	306	NM_006219
p1G4	SLC5A3	Solute carrier family 5, member 3	307	AAC39548	308	AF027153
p1G14	Cytohesin binding protein	PSCDBP	309	NP_004279	310	NM_004288
p1A5	SLC2A5	Solute carrier family 2, member 5	311	NP_003030	312	NM_003039
p1A6	SLC2A5	Solute carrier family 2, member 5	311	NP_003030	312	NM_003039
p1B6	Adipophilin	Adipophilin	313	NP_001113	314	NM_001122
p1B7	Adipophilin	Adipophilin	313	NP_001113	314	NM_001122
p1B8	Adipophilin	Adipophilin	313	NP_001113	314	NM_001122
p1B9	Adipophilin	Adipophilin	313	NP_001113	314	NM_001122
p1G17	Early development regulator 2	Early development regulator 2	315	NP_004418	316	NM_004427
p1G3	B-cell translocation gene 1	B-cell translocation gene 1,	317	NP_001722	318	NM_001731
p1F22	Sorting nexin 9	SH3PX1	319	NP_057308	320	NM_016224

p1G12	Cyclin G2	Cyclin G2	321	NP_004345	322	NM_004354
p1F11	Hypothetical protein LOC51754	NAG-5 protein	323	XP_049657	324	AL137430
p1F16	CYP1B1	Cytochrome P450 IB1 (dioxin-inducible)	325	NP_000095	326	NM_000104
p1F14	Butyrate response factor 1	Butyrate response factor 1	327	NP_004917	328	NM_004926
p1F17	P8 protein (candidate of metastasis 1)	p8 protein (candidate of metastasis 1)	329	NP_036517	330	NM_012385
p1C1	CXCR4	chemokine (C-X-C motif), receptor 4 (CXCR4)	331	NP_003458	332	NM_003467
p1C2	CXCR4	chemokine (C-X-C motif), receptor 4 (CXCR4)	331	NP_003458	332	NM_003467
p1F3	Hypothetical protein XP_017131	solute carrier family 16, member 6	333	XP_017131	334	XM_017131
p1F20	Proline-rich protein with nuclear targeting signal	Proline-rich protein with nuclear targeting signal (B4-2)	335	NP_006804	336	NM_006813
p1F6	Hypothetical protein hqp0376	RNA helicase-related protein	337	T08745	338	AF078844
p1F4	CYP1	Cytochrome P450, subfamily XXVIIB, polypeptide 1	339	NP_000776	340	NM_000785
p1F15	SHB adaptor protein	SHB adaptor protein	341	NP_003019	342	NM_003028
p1F13	Papillomavirus regulatory factor PRF-1	Papillomavirus regulatory factor (PRF-1)	343	NP_061130	344	AK023418
p1A7	SLC31A2	SLC31A2/ hCTR1	345	NP_001851	346	NM_001860
p1A21	UDP-glucose pyrophosphorylase 2	UDP-glucose pyrophosphorylase 2 (UGP2)	347	NP_006750	348	NM_006759
p1B4	Proline 4-hydroxylase, alpha polypeptide II	Proline 4-hydroxylase, alpha polypeptide II	349	NP_004190	350	NM_004199
p1B5	Proline 4-hydroxylase, alpha polypeptide II		349	NP_004190	350	NM_004199
p1B10	Stearoyl-CoA desaturase	Stearoyl-CoA desaturase	351	NP_005054	352	NM_005063
p1B11	Stearoyl-CoA desaturase	Stearoyl-CoA desaturase	351	NP_005054	352	NM_005063
p1B12	Stearoyl-CoA desaturase	Stearoyl-CoA desaturase	351	NP_005054	352	NM_005063
p1B13	Diacylglycerol kinase, zeta	Diacylglycerol kinase, zeta	353	NP_003637	354	NM_003646
p1B22	Protease, serine, 11	Serine protease 11	355	NP_002766	356	NM_002775

p1B23	Interleukin 1 receptor antagonist	IL-1 receptor antagonist, alternatively spliced forms	357	NP_000568	358	NM_000577
p1B24	NS1-binding protein	NS1-binding protein	359	NP_006460	360	NM_006469
p1C3	Activin A receptor, type I	Activin A receptor type I	361	NP_001096	362	NM_001105
p1C4	FGF receptor activating protein 1	FGF receptor activating protein 1 (FRAG1)	363	NP_055304	364	NM_014489
p1C5	Galectin 8	Galectin-8	365	NP_006490	366	NM_006499
p1C6	Glucose phosphate isomerase	Glucose 6-phosphate isomerase	367	NP_000166	368	NM_000175
p1C7	D123	D123 protein	369	NP_006014	370	NM_006023
p1C8	DEC-1	Dec1.	371	NP_003661	372	NM_003670
p1C9	RAB-8b protein	Rab-8b	373	NP_057614	374	NM_016530
p1C10	Regulator of G-protein signalling 1	BL34	375	NP_002913	376	NM_002922
p1C11	Polyubiquitin	Polyubiquitin UbC	377	BAA23632	378	AB009010
p1C12	Integrin, alpha 5	Integrin alpha 5	379	NP_002196	380	NM_002205
p1C13	Jk-recombination signal binding protein	Jk-recombination signal binding protein	381	AAA60258	382	L07872
p1C14	Abstrakt	DEAD-box protein abstrakt	383	NP_057306	384	NM_016222
p1C15	High-mobility group protein 2	High mobility group 2 protein	385	NP_002120	386	NM_002129
p1C16	Decidual protein induced by progesterone	Decidual protein induced by progesterone	387	NP_008952	388	NM_007021
p1C19	GM2 ganglioside activator protein	GM2 ganglioside activator protein.	389	NP_000396	390	NM_000405
p1C20	CNOT8	CCR4 associated factor 1 (CAF1)	391	NP_004770	392	NM_004779
	Similar to Nucleoside phosphorylase	Nucleoside phosphorylase	393	None	394	AA430382
p1P5	SCYA2	Monocyte chemotactic protein 1	395	NP_002973	396	NM_002982
p2L23	Endothelin 1	Endothelin 1	397	NP_001946	398	NM_001955
	Similar to Heat shock 70kD protein 4	Heat shock 70kD protein 4	399	None	400	AA633656
p1K9	Lipocortin I	Annexin AI	401	NP_000691	402	NM_000700
p1K23	MYC	p67 myc protein	403	NP_002458	404	NM_002467
p1K15	Alpha-2-macroglobulin	Alpha-2-macroglobulin	405	NP_000005	406	NM_000014
p1K8	SCYA4	Macrophage inflammatory protein	407	XP_008449	408	XM_008449

		1b				
p1M24	Sex hormone-binding globulin	Sex hormone-binding globulin	409	NP_001031	410	NM_001040
p1K7	ATP-binding cassette E1	ATP-binding cassette, sub-family E (OABP), member 1	411	NP_002931	412	NM_002940
p1K16	CCT6A	Chaperonin / Tcp zeta 1	413	NP_001753	414	NM_001762
p1K18	Colony-stimulating factor1	Colony stimulating factor 1 (macrophage)	415	AAA52117	416	M37435
p1N1	GA17	Dendritic cell protein (GA17)	417	NP_006351	418	NM_006360
p1K22	GPR44	G protein-coupled receptor 44	419	NP_004769	420	NM_004778
p1K14	Keratin 6B	Keratin 6A	421	NP_005546	422	NM_005555
p1K13	Lymphocyte adaptor protein	lymphocyte adaptor protein	423	NP_005466	424	NM_005475
p1J20	Neuro-oncological ventral antigen 1	Neuro-oncological ventral antigen 1	425	NP_002506	426	NM_002515
p1J22	Neutral sphingomyelinase (N-SMase) activation associated factor	N-SMase / FAN	427	NP_003571	428	NM_003580
p1K1	Cyclophilin F	Peptidylprolyl isomerase F (cyclophilin F)	429	NP_005720	430	NM_005729
p1K3	Pleckstrin	PLECKSTRIN	431	NP_002655	432	NM_002664
p1J19	CFFM4	High affinity immunoglobulin epsilon receptor beta	433	NP_067024	434	NM_021201
p1K2	CFFM4	High affinity immunoglobulin epsilon receptor beta	433	NP_067024	434	NM_021201
p1K5	Ribosomal protein L36a	Ribosomal protein L44	435	NP_000992	436	NM_001001
p1J17	SLC6A1	Solute carrier family 6 No1	437	NP_003033	438	NM_003042
p1J18	Synaptopodin	Synaptopodin	439	NP_009217	440	NM_007286
p1J15	TERA protein	TERA protein	441	NP_067061	442	NM_021238
p1K4	TSC-22	TGF beta-stimulated protein TSC-22	443	NP_006013	444	NM_006022
p2A14	EST	Tubulin, beta, 2	445	None	446	AA988110
p1J23	Calgranulin A	Calgranulin A	447	NP_002955	448	NM_002964
p1J21	Replication factor C large subunit	Replication factor C (145 KDa)	449	NP_002904	450	NM_002913
p1J24	Signal recognition particle 19kD	Signal recognition particle 19 kD protein	451	NP_003126	452	NM_003135

p1J16	cDNA: FLJ23019 fis, clone LNG00916	Transcription factor SUPT3H	453	None	454	AK026672
p1J2	Proteasome subunit, alpha type, 4	Proteasome component C9	455	NP_002780	456	NM_002789
p1J9	MAFB	Maf-related leucine zipper homolog	457	NP_005452	458	NM_005461
p1J10	DNCLI2	dynein, cytoplasmic, light intermediate polypeptide 2	459	NP_006132	460	NM_006141
p1J1	Chromobox homolog 3	Heterochromatin-like protein 1	461	NP_057671	462	NM_016587
p1J5	SCYA7	Monocyte chemotactic protein 3	463	NP_006264	464	NM_006273
p1J11	Fatty-acid-Coenzyme A ligase, long-chain 2	Fatty-acid-Coenzyme A ligase, long-chain 2	465	NP_066945	466	NM_021122
p1J8	Programmed cell death 5	Programmed cell death 5 / TFAR19	467	NP_004699	468	NM_004708
p1I20	SCYA3L	Small inducible cytokine A3	469	CAA36397	470	X52149
p1J3	Furin	Cytochrome c oxidase subunit VIc	471	NP_002560	472	NM_002569
p1J12	Nuclear autoantigenic sperm protein	NASP histone-binding prot.	473	NP_002473	474	NM_002482
p1I23	Ecotropic viral integration site 2A	Ecotropic viral integration site 2A	475	NP_055025	476	NM_014210
p1J7	Sjogren syndrome antigen B	Sjogren syndrome antigen B	477	NP_003133	478	NM_003142
p1I21	SCYA8	Monocyte chemotactic protein 2	479	NP_005614	480	NM_005623
p1I19	GRO2	GRO2/ macrophage inflammatory protein 2a	481	NP_002080	482	NM_002089
p1J4	Small nuclear ribonucleoprotein D1	Small nuclear ribonucleoprotein SM D 1	483	NP_008869	484	NM_006938
p1I24	GRO1	GRO1/ macrophage inflammatory protein 2 precursor	485	NP_001502	486	NM_001511
p1I18	Selectin L	Lymphocyte adhesion molecule 1	487	NP_000646	488	NM_000655

Sequence listing

1

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2

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7

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8

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9

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